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ASPECTS OF THE PHYSIOLOGY OF GENE ACTION[1]

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WHAT geneticists want to know is what a gene is and how it works. If we knew what a gene is, we probably could find out easily how it works; and if we knew what the mechanism of gene action is, we would probably be able to predict, to some extent at least, its structure. These two possibilities indicate the two main ways of approach to the gene.

One can try a direct attack on the gene from what we may call the gene-end of the chain of reactions connecting the gene with the character. Such studies are under way, in relation with various particular problems. To this group belong studies of gene dosage, studies of the position effect, studies of the process of mutation, studies of the structure of the salivary gland chromosomes, etc.

Studies of the developmental effects of genes, on the contrary, correspond to the second way, starting at the character-end of the postulated chain of reactions. The first thing to do, if we follow this line, is to analyze this chain of reactions and then to try to follow it backwards, toward the gene.

Studies of the first type are commonly considered as more hopeful because they seem to attack something that

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is closer to the gene than the character, with which we necessarily have to start in the studies of the second type. It should not be forgotten, however, that this does not mean at all that the methods implied in the first case are more direct than those in the second; and it should be kept in mind also that the idea that studies of the second group attack something which is very far away from the gene is based entirely on the tacit assumption that the chain of reactions connecting the gene with the character is a very long one. This assumption has certainly some probability of being correct, but it also may be fundamentally wrong.

My purpose to-day is to report on a certain line of work in the field of developmental genetics. As I have said, the most interesting prospect here is the possibility of building up the chain of reactions connecting the gene with the character, this chain being important not only as an eventual indicator of the nature of the gene, but also having a bearing on the general problem of differentiation. From this point of view, the important point was to find some non-autonomous characters, since only with those is it possible to use the most efficient methods of experimental embryology, such as grafting. In Drosophila the first character of this kind—the vermilion eye color—was found years ago by Sturtevant (1920); and in Ephestia, in 1933, by Caspari. Later Beadle and I (1936) found the cinnabar eye color of Drosophila to be equally dependent in its development. Applying the transplantation method Caspari, on one side, and Beadle and I, on the other, were able to demonstrate:

(1) That the mutants considered are characterized by their failure to produce certain substances required for the development of the pigment characteristic of the wild type and that they remain sensitive to these substances;

(2) That their non-autonomous or dependent development is due to the action of these substances in the new environment; and

(3) That, therefore, their autonomous or non-autonomous development is largely dependent on the nature of the environment.

By using the eye colors as examples of characters controlled by genes, and by applying the transplantation method, it was possible to start reconstructing the chain of reactions leading to the pigment formation. A great deal of interesting information has already been obtained. But in this talk I will limit myself to a particular phase of this chain of reactions, namely the diffusible substances which are, as I said, the factors of dependent development.

I will give you first the main facts which have led us to postulate their existence; and I will then try to tell you what we know at the present time about their nature and functions.

The original experiments which led to the postulation of the existence of diffusible substances in Drosophila can be summarized as follows:

If an anlage of a vermilion eye is implanted into a wild-type fly, the implant develops wild-type pigment. Similarly, if a cinnabar eye is implanted into wild type, the implant develops wild-type pigment. Since the implant lies freely in the body cavity of the fly, it is clear that the change of pigmentation must be brought about by some product circulating in the lymph of the host. The question arises whether there is only one, or two different substances. The reciprocal transplants between *v* and *cn* give the following results:

v in *cn*: the implant is +
cn in *v*: the implant is *cn*

We must therefore conclude that the lymph of the wild-type host contains two different substances, the cn^+ and v^+ substances; and that the first of these substances is lacking in *cn* flies, while both of them are lacking in the mutant vermilion.

At the present time the correctness of this interpretation has been directly proved by methods of chemical extraction (Khouvine, Ephrussi and Harnly, 1936; Thimann and Beadle, 1937).

In the preceding examples of transplantation experiments, the effect is from the host on the implant. Under certain conditions the opposite effect can be observed. If, for example, a *cn* eye is implanted in a $w^a v$ (apricot vermilion) fly, the eyes of the host are modified in the direction of the w^a (apricot; not-vermilion) phenotype. This effect is interpreted as being due to the release by the implant of the v^+ substance. It should be noted here that in this case the *cn* eye serves as a source of the substance, while in the experiments described above it served to detect the presence of another substance. An analogous situation will be met with in the experiments on Ephestia, where also the same organ can serve the two purposes.

In Ephestia we will have to consider several characters under control of a single gene. The wild race and a red-eyed mutant found by Kuhn and Henke (1930) can be characterized as follows:

	Wild race (*AA*)	Red-eyed mutant (*aa*)
Imaginal eyes	Black	Red
Color of testes	Brown	Colorless
Larval eyes	Heavily pigmented	Little pigment
Larval skin	Red	White transparent
Brain	Dark brown	Pale

It was found by Caspari (1933) that if a testis of the wild race is implanted into a red-eyed larva, the color of the host's testes is modified; and that if a testis from a red-eyed animal is implanted into a wild-type host, the color of the implant is modified toward that corresponding to the wild phenotype. Later Kuhn and his co-workers showed that the whole set of sensitive organs (imaginal eyes, testes, larval eyes, brains, skin of the larvae) can be affected by the implantation of wild-type testes and

other organs. The substance responsible for all these changes has been called by Kuhn, Caspari and Plagge (1935) the A-hormone.

Knowing these elementary facts, we will now consider some questions concerned with the production of the active substances.

First, where are they produced?—In both species the active substances are produced in specific places, but the organs producing the v^+ and cn^+ substances in Drosophila and the A-hormone in Ephestia are different. Only the eyes produce active substances in both species. In addition to them, in Ephestia the testes, the ovaries and the brains produce the A-hormone. The ovaries, the testes, and the brains of Drosophila, on the contrary, do not produce detectable amounts of either the v^+ or the cn^+ substances.

In Drosophila, Malpighian tubes also produce both the v^+ and the cn^+ substances, while the fat bodies produce the v^+ substance alone. Whether or not the last two organs of Ephestia produce the A-hormone is not known.

It is clear, in spite of the incompleteness of these data, that the distribution of the hormone-releasing organs is not the same in the two species considered. On the other hand, it is interesting to observe that in both species there is more than a single organ producing and releasing the hormones; this is a situation very different from the one we are familiar with in the case of vertebrate hormones.

Considering next the time of production of the active substances we can say that the developmental stage at which the v^+ and cn^+ substances of Drosophila are produced does not seem to be the same for the different organs producing the substances.

Both substances are present in a high concentration in the lymph of wild pupae and, probably in a lower concentration, in the lymph of larvae during all or part of a 24-hour interval just prior to puparium formation. In the last quarter of the pupal development the concentration

of both v^+ and cn^+ substances falls again; presumably part of each substance is used and the production of new substance goes on at a lower rate.

The injection of extracts of Malpighian tubes from wild type larvae 24 hours after hatching from the eggs shows, as demonstrated by Beadle (1937), the presence in these organs of both v^+ and cn^+ substances at this early stage. Injection of extracts of wild type larval fat bodies gives, on the contrary, negative results: presumably the production of v^+ substance (cn^+ substance is never produced by fat bodies) starts in these organs much later than in the Malpighian tubes. No precise data are available as yet concerning the time of production of the active substances by the eye.

In Ephestia, the A-hormone is concerned, as we have already seen, with the differentiation of some larval characters as well as with that of certain adult characters. It is clear, therefore, that it must appear in the lymph of wild-type animals in the larval stage.

Plagge (1936) has shown that if testes from very young wild-type larvae are implanted for a period of two days into nine-days old pupae of the red-eyed race, and then taken out, there is an effect on the host's eyes. This experiment shows that the A-hormone is actually produced at a sufficient rate by the testes of very young animals. On the other hand, the brain produces the A-hormone in the larval and not in the pupal stage.

Concerning the minimum time necessary for the production of an effective amount of active substance, some information is provided by experiments on Ephestia. Plagge (1936) has implanted testes from larvae of the wild race into pupae of the red-eyed race and has withdrawn the implants after various periods of time. If the implants are taken out 12 hours after implantation, no visible effect on the eye color of the host is produced. After an action of 16 to 19 hours, part of the hosts show an intermediate, brown eye color. After 24 hours, some

hosts have black eyes, corresponding perfectly to the wild phenotype. After 48 hours 50 per cent., and after 72 hours the great majority, of the hosts show a complete change towards the wild phenotype.

It is clear from these experiments that in 24 hours an effective amount of the A-hormone is released by the testis.

Turning to the problem of utilization of the active substances we must first remark that in Drosophila, as well as in Ephestia, the active substances are not necessarily used as soon as they are produced and released in the blood stream. They can be stored.

In Drosophila, Beadle (1937) has shown that if *v* Malpighian tubes are implanted into wild-type hosts, they will take in a certain amount of v^+ and cn^+ substances, which can then be extracted from them.

Another example of storage without immediate utilization is the phenomenon described by Kuhn, Caspari and Plagge (1935) and by Kuhn and Plagge (1937) as "Predetermination." If an Ephestia ♀ heterozygous for *A/a* is backcrossed to an *aa* ♂, it is found that the homozygous *aa* offspring are matroclinous with regard to two larval characters—the color of the larval skin and that of the larval eyes. The characters of the adults are not affected, however, and correspond to those of the red-eyed race. That this "predetermination" is actually due to the action of the A-hormone of the heterozygous mother on the egg is demonstrated directly by transplantation experiments. If testes from the wild race are implanted into ♀♀ larvae of the red-eyed race and if the resulting moths are crossed to red-eyed ♂♂, the progeny will show the wild phenotype in the larval stage (dark larval eyes).

It is clear that the A-hormone released by the implanted wild-type testis has been absorbed by the egg, stored and made use of much later, in the process of pigment formation.

In both species the hormones considered work at specific times or developmental stages, called "sensitive" or "effective" periods.

Let us first consider the situation in Drosophila. Body fluid from wild-type pupae, known to contain the v^+ and cn^+ substances, injected into w^a v hosts (sensitive to the v^+ substance) of various ages (larvae or pupae), produces a strong change of the host eyes toward the w^a phenotype, if the injection is made not later than 65 hours after puparium formation (at 25° C.). If the injection is made later, only minor, insignificant modifications are produced (Beadle, Clancy, and Ephrussi, 1937).

Similarly, injections of wild-type pupal body fluid into w^a; *cn* hosts, sensitive to the action of the cn^+ substance, made at various developmental stages, are effective if made not later than 67 to 68 hours after puparium formation (Harnly and Ephrussi, 1937).

It can be concluded that the major "effective periods" for the action of these substances *end* at this time which corresponds to 0.7 of the pupal development and immediately precedes the onset of the visible pigment formation in the eyes of *v* and *cn* flies. These experiments, indicating the end of the sensitive periods, do not, however, give us any information about their beginning. As we have seen, the substances injected may be stored and used later.

In Ephestia, Plagge (1936) has studied the sensitive periods of the imaginal eyes and testes of the red-eyed race.

In so far as the eye is concerned, it has been found that the eyes are changed to the wild phenotype if a wild-type testis is implanted into red-eyed pupae before the 9th day after puparium formation. If the implantation is made on the 10th or 11th day, the host eyes undergo only a partial change and become intermediate. The result is completely negative if a testis is implanted on the 12th day after puparium formation.

This result can of course be interpreted in different ways. It can be assumed that there is a sensitive period

which ends between the 11th and 12th days; or that the time between the implantation and eclosion (23rd day) is too short for the pigment deposition; or, finally, that the time is too short for the production of a sufficient amount of the hormone. But Plagge has shown that, in the wild race, the pigment is normally formed in the course of the first 10 or 11 days; and, in the red-eyed animals with wild type testes implanted on the ninth day, the full color develops two days after the implantation. Consequently, the pigment formation goes on very quickly. Therefore, we have to conclude that the time factor does not constitute a source of error in the interpretation postulating a sensitive period. For the eye then the sensitive period ends when about 50 per cent. of the pupal development is completed. We have already seen that in Drosophila it ends later, in terms of per cent. of development.

The simple consideration of the fact that there are also larval organs in Ephestia which are affected by the A-hormone shows that the effective periods of these organs must occur earlier in development than that of the imaginal eyes. But even within the imaginal organs the effective periods do not coincide in time. The implantation of wild-type testes into red-eyed larvae leads, as we have seen, to the development by the hosts' testes of a color characteristic of the wild phenotype. If, however, the implantation is made into pupae, the hosts' testes are not modified. Therefore it can be concluded that the sensitive period for the testes pigmentation ends just prior to puparium formation (Plagge, 1936).

In addition, it appears that even within a single organ —the eye—the reactivity of different components is different. In the eye of the adult moth of the wild type the main pigment is concentrated in two zones: one is the sheet of retinular cells and the other, deeper, is in immediate contact with the optic ganglion. The color normally appears first in the external, retinular sheet and then proceeds toward the deeper parts.

If a wild-type testis is implanted into a larva of the red-eyed race and withdrawn after puparium formation, the main part of wild-type pigment develops in the external, retinular zone. If, on the contrary, the implantation is made in the middle of the pupal development, most of the pigment develops in the nervous, deeper portion. Obviously, at this stage, the retinular cells have already lost their sensitivity to the A-hormone, while the cells of the nervous part remain sensitive (Plagge, 1936).

Considering the properties of the active substances, we will note that the molecules of the Ephestia hormone, as well as those of the v^+ and cn^+ substances of Drosophila, are probably quite complicated structures. This follows from the fact that, in both cases, what are probably very small changes or rearrangements are followed by very serious modifications of their specific effects. In Ephestia, for example, the hormones produced by the testes or the ovaries of the wild race on one hand, and the hormone produced by the brain on the other, seem to be qualitatively different (Kuhn, 1936). If the effects of implanted testes and ovaries of the wild type on red-eyed hosts are compared, it is found that there is a strong correlation between the pigmentation of the hosts' testes and eyes—the stronger the effect on the testes, the stronger it is on the eyes. But such a correlation does not exist if the effect is produced by implanted brains. If two or three brains are implanted, the maximum effect on the eyes can be obtained (black eyes, corresponding to the phenotype of the wild race), and yet there is no modification of the color of the testes, which remain pigmented as in the mutant controls. The absence of an effect of the implanted brains on the testes color can not be due to some time relationship, because we have seen that the brain-hormone is produced in the larval stage and that the testes are sensitive to the A-hormone until a stage just prior to puparium formation. It seems clear, then, that the two hormones—that produced by the gonads and that produced by the

brain—are qualitatively different; and yet they must be very closely related since they both produce the same effect on the eye-pigmentation. Undoubtedly, this means also that the pigment-forming system is not exactly the same in the eye and in the testes; and a very simple hypothesis, postulating a supplementary factor in the reaction, would account for the situation.

The whole situation is similar in Drosophila. There is a great deal of evidence showing that the v^+ and cn^+ substances are very closely related (Beadle and Ephrussi, 1936, 1937; Ephrussi and Beadle, 1937). It has been found, for example, that in certain mutants the cn^+ substance may be lacking, while the v^+ substance is present; but whenever a mutation leads to the complete lack of v^+ substance, the cn^+ substance also disappears. There is much evidence to show that the v^+ substance can be converted to the cn^+ substance. That is why we have postulated that both substances are formed in a common reaction, the first link of which is the v^+ substance and the second, the cn^+ substance:

$$\longrightarrow v^+ \longrightarrow cn^+$$

The v^+ substance probably serves as precursor for the cn^+ substance and through a small change in structure is converted to the cn^+ substance. We have no evidence of the reversibility of the transformation: $v^+ \rightarrow cn^+$. The solubilities of both substances are very similar and the chemical work, fractioning of extracts, has so far not permitted the separation of them. But the small difference between the two substances is very specific. The v^+ substance will not transform the *cn* pigment. Experiments involving injections of extracts of *cn* pupae produce no effect on *cn* flies, while producing strong changes in *v* flies (Thimann and Beadle, 1937); and we know that specific genes are concerned in a highly specific way in blocking the reaction leading to the formation of these substances at different levels.

From what has been said about the small differences between the hormones released by the brains and testes of Ephestia, or between the v^+ and cn^+ substances of Drosophila, we naturally conclude that these substances are highly specific in their effects. But on another side, it has been found (Ephrussi and Harnly, 1937, Kuhn and co-workers, Beadle) that the substances considered have quite a wide distribution among different groups of Insects. We have found, for instance, that the v^+ and cn^+ substances of Drosophila are present in extracts from Calliphora (Dipteran) and Galleria (Lepidopteran). Unpublished results of Beadle, Maxwell and Anderson indicate that the v^+ and cn^+ substances are also present in the wasp Habrobracon; and—this is a fact of special interest—it appears that extracts of Ephestia testes contain both active substances of Drosophila. Dr. Beadle has further found the two substances in the crab Uca, indicating that the substances are not limited to the insects.

Similarly, the A-hormone of Ephestia has been detected by Plagge (1936) in several other Lepidoptera (Laspeyresia, Plodia, Plusia, Acidalia) and in Galleria melonella, which, as I said, also contains the two active substances of Drosophila.

All these facts show that these substances, while very specific in stimulating a definite reaction in definite cells, are not "species-specific." Such a behavior is quite characteristic of the hormones known in vertebrates.

The next important fact concerning the mode of action of the active substances is that, in Drosophila as well as in Ephestia, their effects are, within certain limits at least, proportional to their concentration.

In Ephestia this has been established by Kuhn's student, Da Cunha (1935), and by Kuhn, Caspari and Plagge (1935). Here the implantation of one ovary of the wild race into a red-eyed animal does not lead to the formation of black eyes, like those of the wild race. The amount of A-hormone released is too small and the result is an inter-

mediate, brown eye color. But the implantation of two ovaries increases the effect and leads to the development of dark brown or black eyes.

In Drosophila, similar facts have been established by Beadle (1937) who has shown that wild-type Malpighian tubes release the v^+ and cn^+ substances. Implantations of one, two, three or four Malpighian tubes produce effects on the color of the host eyes which are of increasing strength.

Finally, in our work on extraction and purification of the v^+ and cn^+ substances, we have had the opportunity to see that the intensity of the reaction is, roughly speaking, directly proportional to the concentration of the active substances.

In connection with the problem of the mode of action of the active substances, the question as to whether they merely assist the reaction or whether they really participate in it is raised.

If we take a wild-type eye of Drosophila as an example of a hormone- and pigment-forming organ, a very interesting relation can be shown between the release of the hormone and the pigment formation. It can be shown that the eye will not release the hormone as long as its own requirements are not satisfied. The following experiment will illustrate this relation.

A wild-type eye implanted into a w^a;*cn* host modifies the host's eyes toward the w^a phenotype, which means that the implant releases the cn^+ substance. If the eye produced an amount of the cn^+ substance greater than that required for its own pigment formation, it should also release it when implanted into a *v* host, unable to supply the implant with the v^+ substance. But the experiments show that when a wild-type eye is implanted into a *v*;*cn* eye together with a w^a;*cn* eye (serving as a detector of the cn^+ substance released), this second implant is not modified and the wild type eye itself remains a little lighter than the wild-type control eyes implanted into wild-type larvae. It

is clear, then, that the wild-type eye does not produce enough of the active substance and utilizes the entire amount for its own needs.

If now a wild-type fat body, known to release the v^+ substance, is implanted in the same *v;cn* host, together with the wild-type and the w^a*;cn* eyes, then a clear effect on the w^a*;cn* eye is observed; and at the same time one can see that the wild-type eye has developed its normal pigmentation (Ephrussi and Chevais, 1937).

This shows that, as I have said, the eye does not release the substance as long as it has not covered its own needs. There is no free, random diffusion of the hormone molecules; the hormone is preferentially absorbed by the eye itself, which probably means that it enters a stable combination with another substance in the same cell where it was formed. In other words, it participates directly in the pigment-forming reaction.

This "priority effect," as we may call it, does not appear as clearly in Ephestia. For example, testes of the black-eyed *AA* race, implanted into a red-eyed *aa* animal, do not develop the full amount of pigment. And yet there is a marked effect on the host's eyes, which shows that the implanted testis has released a certain amount of A-hormone. The results obtained by the implantation of wild-type brains are similar. One is inclined then to conclude that, contrary to the release of active substances by the Drosophila eye implant, in Ephestia the hormone-forming organs do release the hormone before covering their own needs. But the situation is complicated here by the fact that in control experiments, involving the implantation of testes from wild type into wild animals, the implants do not develop a full pigmentation either. They do develop more pigment than in red-eyed hosts, but less than they would have developed in their normal position. These facts show that the lesser pigmentation of the wild implants in mutant hosts is due partly to the operation itself, and partly to the nature of the host. But this

makes it very difficult to decide whether or not what I have called the priority effect occurs here.

But other facts show that in Ephestia also the A-hormone enters into a very stable combination. It has been found by Kuhn's group that the three organs which release the hormone—the testes, the ovaries and the brain—may be arranged in the following order with regard to the amount of A-hormone released:

Testis > ovary > brain

This can be concluded from experiments involving the implantation of these organs taken from wild-type animals into red-eyed hosts. In such an experiment it is found that, while testes implantation affects the color of the host's (1) adult eyes, (2) larval skin, (3) testes, and (4) larval eyes, the implantation of ovaries affects only the color of the larval eyes and the testes; and that implanted brains affect only the pigmentation of the larval eyes. But the difference is not only in the number of organs affected, but also in the intensity of the effect. If the color of the adult's eyes is taken as a criterion, it is found that similar effects are obtained by the implantation of one testis, or two ovaries, or two or three brains. Now, while one implanted ovary usually grows and differentiates normally, a single ovarian tube implanted under the same conditions degenerates and is usually resorbed. The unexpected thing here is that the implantation of a single ovarian tube, followed by the resorption of the implant, produces a strong effect on the host—much stronger than the implantation of a whole ovary. Obviously, the degenerating ovary gives up the substance that it would not give up in its normal condition. In other words, in the normal cell the hormone must have entered a stable combination. It must be noticed that the implantation of a single ovarian tube from the mutant red-eyed race does not lead to the same result. Therefore, the effect can not be attributed to some incidental product of cell degeneration (Kuhn, 1936).

What we are naturally particularly anxious to know is how close the substances, which we can consider as an intermediate link between the genes and the characters, are to the genes. So far we can, unfortunately, use only indirect evidence.

According to Kuhn, Caspari and Plagge (1935) in the hormone forming cells of Ephestia, the production of the A-hormone is not the first and direct action of the *A* gene. This conclusion is drawn from the relationship between dominance and quantitative action of the hormone; action which is, as demonstrated, within certain limits at least, directly proportional to the amount or to the concentration of the hormone. The response of the pigment cells is more or less pronounced, according to the quantity of hormone supplied; in other words, it is not an "all or none reaction." As I have already shown, the implantation of two brains, in Ephestia, gives a stronger effect than the implantation of a single one. But, on the other hand, an ovary from a heterozygous (*A/a*) animal produces an effect equal to that of a homozygous (*A/A*) animal. The effect obviously depends on the amount of tissue, and not on the number of *A* genes in the hormone-producing cells. Therefore, one *A* gene does not produce a definite "quantum" of hormone; it merely determines a certain degree of a "primary reaction," which is not increased by the presence of a second *A* gene. According to Kuhn and his co-workers, the process of dominance is concerned precisely with this primary reaction in the hormone-producing cell.

This reasoning can naturally be applied equally well to the cases of hormonal actions in Drosophila. Here also, in so far as I can see, no difference can be found between hormone producing tissues from homozygous or heterozygous donors.

But the question must be raised: is it, in a general way, necessary to assume a primary reaction? It has to be kept in mind that the genes which we call dominants, in

all these cases, are those that have a positive action—their presence is necessary for the production of a hormone—while those which we call recessives are those which fail to produce the hormone. Therefore, the explanation of the phenomenon of dominance itself does not require a supplementary mechanism. In the cases considered, it fits perfectly the scheme of dominance postulated by Wright (1934). It remains to be seen, then, if the fact that a heterozygous tissue, having only one dominant gene in each cell, produces an effect quantitatively equal to that of a homozygous tissue, having two active genes in each cell, constitutes a critical argument in favor of the existence of what Kuhn, Caspari and Plagge call a primary reaction. Personally, I do not have any theoretical objection to such a view; nevertheless I want to point out that other explanations might be offered for the same facts. It is perfectly possible, for example, that, in a given cell, the amount of elementary materials to be converted to hormone through the action of genes might be limited independently of its genetic constitution. It only needs to be assumed, then, that a single gene has the capacity to transform into hormone the total amount of elementary materials. No increase of the amount of hormone produced will result from the introduction of a second active gene. This interpretation is essentially similar to that offered by Haldane (1930) in connection with the general discussion of the problem of dominance.

For all these reasons, I do not consider the existence of the primary reaction postulated by Kuhn, Caspari and Plagge as demonstrated.

A few words now about what is known concerning the chemical nature of the substances studied.

The study of the v^+ and cn^+ substances of Drosophila has recently been begun. It has been found so far that they are not enzymes; they are heat-stable. They are not proteins in the ordinary sense, since they can be extracted in alcohol-ether mixtures or in alcohol and since they are

dialysable. They are not fats or sterols, since they are not extracted in ether and are soluble in water. Further fractioning indicates that they are not phosphoaminolipides and suggests that they belong to the group of aminobases. Their molecule is rather small, since the active extracts can be dialyzed. All the active extracts, in the present stage of purification, are very rich in nucleic acid, which possibly means that they are closely related to the chromosome.

Concerning the chemical nature of the Ephestia A-hormone still less is known. A short note published recently by E. Becker in *Naturwissenschaften* indicates that this substance also is not a protein nor a fat.

Summing up the results to which I have drawn your attention, we can say that in the cases studied the genes produce their effects by means of special substances or hormones, produced in specific organs at specific times. These substances, highly specific in their effects—but not species specific—affect definite organs at definite developmental stages. The effects of these substances are proportional to their concentration. The substances are not merely conditions of definite reactions, but actually participate in these reactions. Their chemical study, just started, shows that they are not enzymes, not fats, and not proteins in the ordinary sense. It is possible that they are very closely related to the constituents of the chromosomes.

Before I finish, I want to make just one more remark. As I said at the beginning, the cases of non-autonomous development are exceptional. It may appear, then, that what we have studied in Drosophila and in Ephestia are some very special cases having nothing to do with the normal way in which genes usually produce their effects. Personally, I do not believe so. It seems to me that there is no reason to assume that the reactions occurring within the cell in the case of autonomous characters belong to a type essentially different from the one we have studied here.

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A NOTE ON THE SIZE AND COMPOSITION OF OLD *TRIBOLIUM CONFUSUM* POPULATIONS[1]

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THERE exist in the literature few records that merely picture, in quantitative terms, the composition and size of unmolested animal populations which are (1) of considerable age, (2) of known initial density and (3) have been run under controlled environmental conditions. Despite their purely descriptive nature these are data of basic importance in population studies. This point is gaining recognition by the field ecologists, who are more and more taking into account the idea of numbers as well as species in analyzing the biotic community. It has been most completely attacked, however, by the students of human populations who routinely accumulate an amazing body of demographic data concerned with such matters (for a recent survey of this point see Pearl, 1937). The present paper attempts to contribute to this problem by analyzing the composition of year-old populations of the flour beetle, *Tribolium confusum* Duval. This beetle has proved useful in experimental population studies. This is due, in part, to the fact that the entire life-cycle is passed in flour, and the beetles, along with their immature stages, can be removed from the medium by sifting. It is thus possible to take a complete census of a *Tribolium* population without great difficulty. For discussions of the general biology and husbandry of this beetle the papers of Good (1936) and Park (1934a) should be consulted.

In an earlier report (Park and Woollcott, 1937) the oviposition of *Tribolium* in various concentrations of conditioned flour (*i.e.*, flour produced by the activity of the

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beetles living in it) was studied. At the conclusion of this 60-day experiment a series of 200 cultures of known experimental history remained. These cultures were put aside under controlled environmental conditions (in darkened incubators maintained at 28° C., and approximately 40 per cent. R.H.) without any outside interference or renewal of medium for one year. Due to the time required to examine these bottles this "year" does not mean an exact 365 days but refers rather to a span extending from 350 to 370 days. At the time of examination of the cultures the following records were obtained for each bottle: (1) number of eggs; (2) number of larvae; (3) number of pupae; (4) number of living imagoes; (5) number of dead imagoes (not including beetles that were dismembered), and (6) weight of the flour. Each bottle contained at the start of the experiment two pairs of adult *Tribolium* of similar age and genetic history in 32 gm of flour. There were no eggs, larvae or pupae in the cultures at this time. According to the type of flour initially present in the bottles the following experimental series can be recognized:

(1) The Initially Fresh Series: started with 116 bottles containing fresh flour exclusively.
(2) The Initially Lightly Conditioned Series: started with 36 bottles containing fresh flour mixed with from 5 to 10 per cent. (*i.e.*, some bottles contained 5 per cent. and others 10 per cent.) of conditioned flour.
(3) The Initially Medially Conditioned Series: started with 36 bottles containing fresh flour mixed with from 15 to 25 per cent. (*i.e.*, some bottles contained 15 and others 20 and 25 per cent.) of conditioned flour.
(4) The Initially Heavily Conditioned Series: started with 12 bottles containing conditioned flour exclusively.

The results obtained by taking a census of the four groups of *Tribolium* populations after one year of growth are summarized in Tables I and II and graphically represented in Fig. 1. The statistical comparisons mentioned in the text were made by computing the ratio of the difference between selected means to the probable error of that difference.

A number of inferences can be drawn from these data,

TABLE I

STATISTICAL SUMMARY OF (1) NUMBER OF EGGS, (2) NUMBER OF LARVAE, (3) NUMBER LATION AND (6) WEIGHTS OF FLOUR FOR POPULATIONS OF *Tribolium* ONE

Series	Eggs				Larvae				Pupae		Living Imagoes			
	Total	Mean per bottle	σ (eggs)	C.V. (per cent.)	Total	Mean per bottle	σ (lar.)	C.V. (per cent.)	Total	Total	Mean per bottle	σ (imag.)	C.V. (per cent.)	
Initially Fresh flour	567	4.9± 0.25	4.1± 0.17	83.6 ±3.69	2618	22.6± 0.72	11.6± 0.50	51.3± 2.26	23	6954	59.9± 1.10	17.7± 0.77	29.5± 1.30	
Initially Lightly condit. flour	243	6.7± 0.83	7.4± 0.58	110.4 ±8.77	786	21.8± 2.34	20.9± 1.65	95.8± 7.61	3	863	23.9± 1.46	13.0± 1.03	54.4± 4.32	
Initially Medially condit. flour	182	5.1± 1.03	9.2± 0.72	180.3 ±14.3	292	8.1± 1.52	13.6± 1.07	167.9 ±13.3	0	442	12.3± 1.19	10.6± 0.84	86.1± 6.84	
Initially Heavily condit. flour	0				0				0	0				

both in respect of the actual number of beetles as well as their variability. Considering first the total population size for all four experimental series it is patent that there still remains a real differential effect of conditioning even after a year's growth period. Thus the initially fresh series is significantly larger, as regards the total population, than the conditioned series, and, of the latter, the lightly conditioned is larger than the medially conditioned. The populations started in heavily conditioned flour have become completely extinct. These effects are comparable with those described in earlier analytical studies (Park, 1934; 1935; 1936; Park and Woollcott, 1937) in that the cultures containing the most fresh flour are the largest. In a sense the earlier results are extended by the present data. The former merely demonstrated the effect of conditioning on fecundity and fertility, while the latter are concerned with the entire composition of mature populations.

Since none of the cultures were observed during the course of the year it is impossible to conclude what stage

TABLE I

OF PUPAE, (4) NUMBERS OF LIVING AND DEAD IMAGOES, (5) SIZE OF TOTAL LIVING POPU-YEAR OLD AND STARTED INITIALLY IN FOUR DIFFERENT TYPES OF FLOUR

Total living population				Dead imagoes				Flour weight				Number of bottles
Total	Mean per bottle	σ	C.V. (per cent.)	Total	Mean per bottle	σ (imag.)	C.V. (per cent.)	Total	Mean bottle weight	σ (gm.)	C.V. (per cent.)	
10162	88.6± 1.08	17.4± 0.76	19.6± 0.86	3707	31.9± 0.75	12.1± 0.53	37.9± 1.67	2477	21.3± 0.13	2.2± 0.09	10.3± 0.45	116
1895	52.6± 3.45	30.7± 2.43	58.3± 4.63	1009	28.0± 1.02	9.2± 0.72	32.8± 2.60	853	23.7± 0.29	2.6± 0.20	11.0± 0.87	36
916	25.4± 2.88	25.7± 2.03	101.1 ±8.04	1005	27.9± 0.74	6.7± 0.52	24.0± 1.90	888	24.7± 0.17	1.6± 0.12	6.5± 0.51	36
0				120	10.0± 0.68	3.5± 0.48	35.0± 4.82	302	25.1± 0.36	1.9± 0.25	7.5± 1.05	12

of growth the populations were in at the time of their examination. Prior to this time all series had reached, presumably, a greater total size. This, however, can not be considered a proved point until further experiments are performed. The present study suggests that it would be highly desirable to have for *Tribolium,* as well as for other organisms, a quantitative description of the entire life-history of populations in which the flour was not altered. This would provide a basis for future work by opening up new problems and would aid in understanding population dynamics generally. Chapman (1928) has presented some valuable data on the growth of *Tribolium* cultures, but in this work the medium was renewed frequently and the populations not allowed to go into a normal decline. Gause (1931), using Chapman's data, showed that the growth of *Tribolium* populations are adequately described by the logistic curve.

The statistical constants of Table I suggest that the variability is much greater for the conditioned than for the fresh series. The coefficients of variation increase mark-

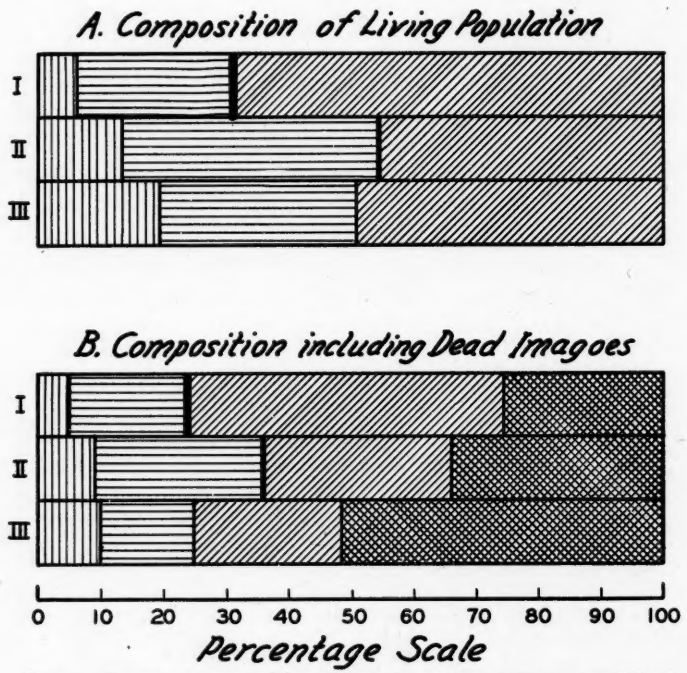

FIG. 1. Percentage composition of eggs, larvae, pupae imagoes and dead imagoes in *Tribolium* populations in three initially different stages of conditioning.

I = Fresh, II = Lightly Conditioned and III = Medially Conditioned cultures. Vertically ruled bars are eggs; horizontally ruled bars are larvae; solid bars are pupae; oblique bars are living imagoes and cross-hatched bars are dead imagoes.

edly as the flour gets more conditioned. There is also the interesting suggestion that the total population is, by and large, less variable in size than the component members of that population. This holds especially true for the initially fresh series where the variation increases progressively from total number on down through imagoes, larvae and eggs. It is also partially true for the initially conditioned groups in that the coefficients of variation for the egg and larval populations are higher than those for imagoes and totals.

The idea that the components of a population may ex-

hibit a characteristic range of variation is deserving of more attention than it has had. It is common parlance to think of populations in terms of their actual census counts, but not nearly as common to appreciate that each component may be distributed about its mean in a distinctive manner. If this variation is a real and repeatable fact a study of it should unearth data important in the understanding of population dynamics.

In considering the three population series in terms of their components (eggs, larvae, pupae and imagoes) a number of instructive conclusions can be drawn. In the first place, a contrast is seen between the initially fresh and the conditioned cultures. The former—"younger" in the sense that they are larger—are predominantly imago populations. The adult *Tribolium* account for approximately 68 per cent. of the total; the larvae 25 per cent. and the eggs 6 per cent. The number of pupae is extremely small: this, however, is characteristic for all three series. In the two conditioned groups the imagoes constitute only about half of the total population, and there has been a corresponding increase in the relative number of eggs and larvae. As seen from Table II and Fig. 1 the eggs rise to a point where they account for about one fifth of the entire population. However, this can not be looked upon as a very consistent figure, due to the extreme variability with which eggs, and to a lesser degree larvae, are found in the conditioned cultures.

If it be characteristically true that the relative number of eggs is greater in older and run-down cultures than in young cultures a puzzling point is raised. Experimental studies have all shown, as a physiological fact, that as the flour gets more conditioned the fecundity of young beetles living in it lowers. This suggests that there should be fewer eggs in the conditioned than in the fresh populations, which is contrary to the results summarized in Table II. It is not apparent at the moment what is behind this interesting discrepancy, although the following possibilities suggest themselves: (1) that a relatively high

concentration of eggs in old cultures is not necessarily characteristic—this explains away the present data on the grounds of excessive variation in egg numbers between bottles; (2) that the egg cannibalism by imago beetles so lowers in conditioned cultures that the drop in fecundity is more than compensated for, and (3) that the fecundity of old *Tribolium* behaves differently relative to conditioned flour than does that of young beetles such as those used in the experimental studies.

As indicated earlier, the number of dead imagoes as well as the living beetles was recorded at the time of examination. These records, when added to those already discussed, suggest several points. It is apparent that the dead beetles accumulate in the populations with considerable consistency. There are significantly more, in terms of total numbers, in the fresh than in the lightly and medially conditioned series, but the mean difference is not large and the variability considerably lower, for example, than that exhibited by eggs and larvae. This is a difficult point to interpret. It suggests that, regardless of the total size of the population, the dead imagoes accumulate to a certain point and then disappear. This is corroborated somewhat by the observation that all the bottles contained dismembered *Tribolium* in various stages of disintegration. Presumably, in dense populations this "saturation" point would be reached quickly; in smaller populations a longer time would be required.

Table I also records the number of dead imagoes found in the heavily conditioned series. Here, the mean is far below that of the other groups. This would be expected on the view that these cultures became extinct at a time when the total population was quite small and when there was probably severe cannibalism between the surviving beetles.

A different picture results when the number of dead imagoes for each series is added to the living population and the whole expressed on a percentage scale. This has been done in Table II and Fig. 1. It is decidedly apparent

that as the environments get more conditioned the relative number of dead *Tribolium* increases. Thus in the initially fresh cultures the dead beetles constitute 27 per cent. of the total population, while in the lightly and medially conditioned cultures they constitute respectively 35 and 52 per cent. of the total. It is difficult to conclude what these figures really mean. They suggest, without critical proof, that the death-rate per unit number of beetles is higher for the conditioned cultures. It is impossible, however, to infer from the results what factor or factors are behind this mortality. This is a problem for future experimentation.

The final point to be made concerns the weight of the medium in the various cultures. At the time of examination it was found that a certain amount of the original 32 gm of flour had been lost. Since none of the cultures were disturbed during the tenure of the experiment it seems safe to attribute this loss to the activity of the beetles themselves. This is supported by several facts. There is a significantly greater decrease in flour weight on the part of the initially fresh series than the others. Likewise, the lightly conditioned group shows a greater drop than does the medially conditioned, with the heavily conditioned cultures displaying the least reduction in flour weight. This would be expected on the view that the concentration of *Tribolium* in the population is responsible for the loss of flour, since this loss is proportional to the total population size. This weight decrease could be due, in part at least, to the fact that the beetles' metabolic waste products—carbon dioxide and water—pass off into the atmosphere. In a certain bottle of the fresh series no living beetles and only four dead imagoes were found. The flour of this bottle weighed 31 gm, which is about 10 gm above the mean. This population obviously became extinct soon after the start of the experiment, and there was little opportunity for the flour to be altered by the beetles. It may be that factors other than the escape of volatile wastes also contribute to the loss of weight.

TABLE II

PERCENTAGE COMPOSITION BY STADIA OF *Tribolium* POPULATIONS

Stadium	Composition of living population (per cent.)						Composition including dead imagoes (per cent.)					
	Initially fresh flour		Initially lightly condt. flour		Initially medially condt. flour		Initially fresh flour		Initially lightly condt. flour		Initially medially condt. flour	
	Per cent. of total	Cumul. per cent.	Per cent. of total	Cumul. per cent.	Per cent. of total	Cumul. per cent.	Per cent. of total	Cumul. per cent.	Per cent. of total	Cumul. per cent.	Per cent. of total	Cumul. per cent.
Egg	5.6	5.6	12.8	12.8	19.9	19.9	4.1	4.1	8.4	8.4	9.5	9.5
Larval	25.7	31.3	41.5	54.3	31.9	51.8	18.9	23.0	27.1	35.5	15.2	24.7
Pupal	0.3	31.6	0.2	54.5	0.0	51.8	0.2	23.2	0.1	35.6	0.0	24.7
Imaginal (living)	68.4	100.0	45.5	100.0	48.2	100.0	50.1	73.3	29.7	65.3	23.0	47.7
Imaginal (dead)	...	...	...	...	...	...	26.7	100.0	34.7	100.0	52.3	100.0
	100.0		100.0		100.0		100.0		100.0		100.0	

Summary

An analysis of the composition of 200 cultures of *Tribolium confusum* raised in four initially distinct types of flour and allowed to grow unmolested has brought out the following points:

(1) The total population size (*i.e.*, after one year) is in inverse proportion to the initial conditioned flour content of the medium. This difference between the various experimental series is due largely to the number of imago and larval beetles and not to the eggs and pupae.

(2) In all cultures the imagoes display less variability of numbers than the eggs and larvae.

(3) The pupae comprise less than 0.3 per cent. of the entire population.

(4) The total number of dead imagoes is quite similar for each flour series. These dead *Tribolium*, however, are found in relatively greater abundance in the conditioned than in the fresh series.

(5) All the cultures lose weight during the year's growth period. This loss is attributed to the escape of metabolic waste products into the atmosphere. It is shown to vary directly with the total size of the population.

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THE INHERITANCE OF RESISTANCE TO MILDEW

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INTRODUCTION

IN a study of the inheritance of resistance to any disease in plants the three following points are of interest: (1) the number of factors for resistance present in each variety, (2) the identity of each factor, and (3) the effect of each factor acting alone. In the study of resistance to mildew, *Erysiphe graminis hordei,* in hybrids between resistant Hanna and susceptible Atlas, the writer[1] (1935) found that Hanna differs from Atlas in one major factor for resistance to this disease. Later, it was designated as the Hanna factor by Briggs and Barry,[1] because it was different from the factor found in the Goldfoil variety. The latter was named the Goldfoil factor.

In future investigations the Hanna and the Goldfoil varieties will serve as testers for these two factors.

This paper deals with the data from the three resistant varieties, Arlington Awnless, Chinerme and Nigrate, in crosses with susceptible Atlas. Data are also available from hybrids between these resistant varieties and the tester varieties, Hanna and Goldfoil.

MATERIALS AND METHODS

A brief history of the three resistant varieties, Arlington Awnless, Chinerme and Nigrate, is of interest because of the similarity of results obtained with them in this investigation. As will be seen presently, Chinerme and Nigrate might be expected to give similar results.

Arlington Awnless C. I. No. 702, *Hordeum intermedium haxtoni tonsum:* According to Harlan, *et al.* (1925) this

[1] Fred N. Briggs and G. L. Barry, "Inheritance of resistance to mildew, *Erysiphe graminis hordei,* in a cross of Goldfoil and Atlas barley." Unpublished manuscript.

variety probably originated from a cross of Tennessee Winter with Black Arabian.

Dr. G. A. Wiebe, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, supplied the following histories of Chinerme and Nigrate:

Chinerme, C. I. No. 1079 (Black Awnless) *H. i. mortoni nigritonsum.* From Chungking, China. Presented by Mr. E. Carleton Baker, American Consul. Received April 21, 1915. "Barley is not grown to any extent in the vicinity of Chungking, as stated by E. H. Wilson, the botanist, in his book on Czechung. It is only in the mountainous Tibetan borderland that it is largely grown. The Chinese do not care for the meal and the grain is chiefly used for making spirits, and for feeding pigs and other domestic animals" (Baker). Planted at Arlington, Va., 1916.

Nigrate C. I. No. 2444. *H. i. mortoni nigritonsum.* From China, a selection out of Chinerme, C. I. No. 1079, made at Chico, Calif., 1916–17.

When grown at Davis, Calif., there is no apparent difference between Chinerme and Nigrate, and they should probably not be considered different varieties.

There is nothing in the history of Arlington Awnless to indicate any close relationship to the other two varieties. In common with Chinerme and Nigrate it has short awns and belongs to *Hordeum intermedium.*

Mains and Dietz (1930) studied the reaction of Arlington Awnless and Nigrate to their five forms of barley mildew. These two varieties reacted very similarly to all five forms, the greatest difference being a Type 1 reaction with Form 5 for Nigrate and a Type 0 for Arlington Awnless. Mains and Martini (1932) studied the reactions of all three varieties to Forms 1, 2 and 3. The reactions of Chinerme and Nigrate were practically identical and only differed slightly from that of Arlington Awnless. Honecker (1934) found only slight differences in the reaction of Arlington Awnless and Nigrate to the two races of mildew recognized by him. Tidd (1937) found no difference between Arlington Awnless and Chinerme in their reaction to Forms 6 and 7. All three varieties have been highly resistant to Form 3 of barley mildew both in the field and greenhouse at Davis.

The hybrids and parent varieties were grown in greenhouse benches filled with about six inches of soil. Thirty seeds were sown in rows which were thirty inches long and five inches apart. Every fifth row was seeded to Atlas. The young plants were inoculated in the three-leaf stage by dusting with spores from diseased plants grown for that purpose. Form 3 of mildew, which has been used in all our studies, was used in these experiments.

The classification of plants was the same as used in previous investigations (Briggs, 1935).

EXPERIMENTAL RESULTS

The F_2 and F_3 hybrids of the several crosses were grown during 1935, 1936, 1937. The F_2 studies were made at the same time that the F_3 rows were being grown. Seed for the F_3 were grown in the field where the mildew infection was too light to permit a classification of F_2 plants.

The plants were classified as resistant (mildew reading 0, 1 and 2) and susceptible (mildew reading 3 and 4). After the first year, the reading of 3 was dropped and all heavily diseased plants were classed as 4. While it is possible to distinguish between types 3 and 4 where one is dealing with a homozygous group of plants, it is not easy to classify individual plants so closely. Likewise, it is not easy to distinguish between readings of 1 and 2 on individual plants. However, there is a fairly wide and distinct difference between plants classed as 2 and 4.

TABLE 1

THE CLASSIFICATION OF F_3 PLANTS AND PARENTS OF THE CROSSES NAMED, GROWN IN THE GREENHOUSE AT DAVIS, CALIF., 1935–1937

Parent or hybrid	Resistant	Susceptible	Value of P* 15 : 1 ratio
	Number	Number	
Arlington Parent	88		
Chinerme Parent	119		
Nigrate Parent	79		
Atlas Parent		503	
Arlington Awnless × Atlas	403	35	> .3
Chinerme × Atlas	329	15	> .3
Nigrate × Atlas	312	22	> .9

* Values of P taken from Fisher (1932).

The classification of F_2 plants of the crosses with Atlas may be seen in Table 1.

The F_2 data are in satisfactory agreement with the 15:1 ratio showing that the three resistant varieties each differ from Atlas in two major factors. This conclusion is adequately confirmed by the F_3 data which are presented in Table 2.

The class intervals were chosen to represent a difference of one susceptible plant in a population of 29, because there was an average of about 29 plants in F_3 rows. The only genotype which may be distinguished with certainty in Table 2 is the homozygous susceptible one. With 29 plants per row you would expect an overlapping between rows segregating for one factor and rows segregating for two factors. Also you would expect some rows, which were heterozygous for two factors, not to contain a susceptible plant. The expectations for each class are readily obtained from Warwick (1932). The percentages of rows expected in the various classes from the several genotypes may be seen in Table 3.

The correspondence between the values expected and those obtained for the actual crosses can readily be seen in Fig. 1.

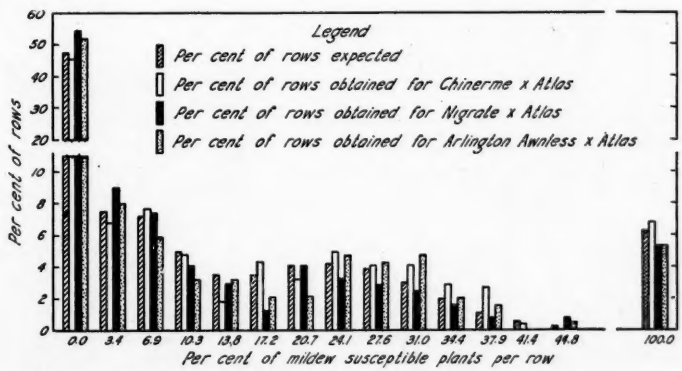

FIG. 1. Comparison of the theoretical frequencies for the classes indicated with those obtained for the three crosses indicated.

TABLE 2

DISTRIBUTION OF PARENTS AND F_3 ROWS OF THE CROSSES NAMED SHOWING THE PERCENTAGE OF MILDEW SUSCEPTIBLE PLANTS IN THE CLASSES INDICATED GROWN IN THE GREENHOUSE AT DAVIS, CALIFORNIA, 1935–1937

Parent or hybrid	Number of rows with the percentage of susceptible plants indicated														Total no. of rows	Value of P 15 : 1 ratio
	0.0	1.7 –5.1	5.1 –8.6	8.6 –12.0	12.0 –15.5	15.5 –18.9	18.9 –22.4	22.4 –25.8	25.8 –29.3	29.3 –32.7	32.7 –36.1	36.1 –39.6	39.6 –43.1	43.1 –46.5	100	
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	Number	
Arlington Awnless	7														221	
Chinerme	16															
Nigrate	9															
Atlas																
Arlington Awnless × Atlas	97	15	11	6	6	4	4	9	8	9	4	3	0	1	10	187	> .5
Chinerme × Atlas	200	30	34	21	8	19	14	22	18	18	13	12	2	0	30	441	> .5
Nigrate × Atlas	133	22	18	10	7	3	10	8	7	6	4	1	0	2	13	244	> .5

TABLE 3

THE PERCENTAGES OF F_3 ROWS EXPECTED IN THE VARIOUS CLASSES INDICATED FOR F_2 PLANTS OF THE GENOTYPES INDICATED

Genotype of F_2 parent plant	Per cent. of F_3 rows with the percentages of susceptible plants indicated :														
	0.0	1.7 –5.1	5.1 –8.6	8.6 –12.0	12.0 –15.5	15.5 –18.9	18.9 –22.4	22.4 –25.8	25.8 –29.3	29.3 –32.7	32.7 –36.1	36.1 –39.6	39.6 –43.1	43.1 –46.5	100
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Homozygous resistant	43.75														
Heterozygous for 2 factors	3.85	7.44	6.94	4.17	1.81	.60	.16								
Heterozygous for 1 factor			.27	.81	1.75	2.91	3.88	4.25	3.90	3.03	2.02	1.16	.58	.25	
Homozygous susceptible															6.25
Total	47.6	7.44	7.21	4.98	3.56	3.51	4.04	4.25	3.90	3.03	2.02	1.16	.58	.25	6.25

Even though the class intervals used are too small for the populations of Nigrate × Atlas and Arlington Awnless × Atlas (that is, too many classes are represented by frequencies below 10, Table 2), the agreement between the expected and actual values is very good.

The data from the crosses of Chinerme, Nigrate and Arlington Awnless with Atlas clearly show that these three resistant varieties differ from Atlas in two independent major factors for resistance to mildew, and that susceptibility is recessive. It is impossible to determine the exact effect of either resistant factor. The presence of a considerable number of plants both in F_2 and F_3 with mildew readings of 1 and 2 suggests either that modifying factors are present or that one of the two factors acting alone may not confer as complete resistance as is found in the resistant parent. A study of these factors in crosses where they occur singly should clear up that point.

The question naturally arises as to whether the two factors found in Chinerme, Nigrate and Arlington Awnless are the same and further if they are the same as those already found in Hanna and Goldfoil. Some of the crosses necessary to settle these points are available, and the F_2 data from these may be seen in Table 4.

TABLE 4

THE CLASSIFICATION OF THE F_2 PLANTS AND PARENTS OF THE CROSSES NAMED. GROWN IN THE GREENHOUSE AT DAVIS, CALIF., 1936–1937

Parent or hybrid	Resistant	Susceptible	Value of P 63 : 1 ratio
Arlington Awnless parent	268		
Chinerme parent	204		
Nigrate parent	170		
Hanna parent	83		
Goldfoil parent	88		
Atlas parent		1,414	
Arlington Awnless × Hanna	569	8	>.7
do do × Goldfoil	611	6	>.2
Chinerme × Hanna	630	10	>.9
do × Goldfoil	601	8	>.5
Nigrate × Hanna	617	13	>.2
do × Goldfoil	595	8	>.5
Chinerme × Arlington Awnless	373	0	
Nigrate × do do	300	0	
Nigrate × Chinerme	565	0	

The three resistant varieties crossed with Hanna and with Goldfoil segregate susceptible plants in F_2, indicating that the Hanna and Goldfoil factors for resistance to mildew are not present in Chinerme, Nigrate and Arlington Awnless. Furthermore, the segregation conforms to the 63:1 ratio, as would be expected where one parent contributes two factors and the other parent a third factor.

Chinerme × Arlington Awnless, Nigrate × Arlington Awnless and Nigrate × Chinerme did not segregate any susceptible plants in F_2. One hundred and sixty-eight F_3 rows were grown from the first cross and 176 F_3 rows from Nigrate × Arlington Awnless without obtaining a single susceptible plant. Therefore Chinerme, Nigrate and Arlington Awnless must have at least one factor for mildew resistance in common. The factors for resistance in these three varieties will have to be studied singly in crosses in order to determine if the same two factors are present in each of the above resistant varieties.

SUMMARY

A study was made of the inheritance of resistance to barley mildew in hybrids involving the three resistant varieties: Arlington Awnless, Chinerme and Nigrate.

These three varieties were shown to differ from susceptible Atlas in two independent major factors for resistance to this disease. Susceptibility to mildew was recessive.

Crosses of these three resistant varieties with Hanna and with Goldfoil segregated out susceptible lines, showing that the Hanna factor and the Goldfoil factor were not carried by Arlington Awnless, Chinerme and Nigrate.

Crosses of Chinerme with Arlington Awnless, Nigrate with Arlington Awnless and Nigrate with Chinerme did not produce any susceptible plants, proving that these varieties have at least one factor for resistance to mildew in common.

These factors will have to be isolated and studied singly in order to determine their identity and effect.

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THE DETERMINATION OF STERILITY IN DROSOPHILA MALES WITHOUT A COMPLETE Y-CHROMOSOME

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IT is a remarkable fact that the Y-chromosome of *Drosophila melanogaster* has no fundamental effect on the development of a phenotypically normal individual. However, males which lack either a whole Y-chromosome or certain fractions of it are completely sterile (Bridges, 1916, Stern, 1929). Shen (1932) has shown that spermatogenesis in Y-deficient males of *D. melanogaster* is perfectly normal, as far as a morphological analysis has discerned, and resulting in the formation of fully formed spermatozoa. These gametes, however, instead of becoming motile in the end section of the tubular testis, remain immobile and later on degenerate. The vasa efferentia of such males accordingly are free from functional spermatozoa.

The genetic constitution of the spermatozoa produced in these sterile males is not of such a nature as to bring about degeneration. An XO male produces X- and O-spermatozoa. The first kind is genetically like that of any normal X-spermatozoon. The second kind when formed in an XY male by non-disjunction is also viable (Bridges in Morgan, Bridges, Sturtevant, 1925, p. 115–116; Gershenson, 1933, and others). It might be objected that O-spermatozoa are generally rare in XY males and that only their presence to the amount of one half of all spermatozoa, as in an XO male, causes the degeneration of the whole spermatic content. Such an argument, however, loses its force when males with the Y-chromosome fragment Y″ are considered. Males of the constitution

XY″, like XO males, form only immobile spermatozoa, which degenerate later. Here half of the spermatozoa formed possesses the X- and half the Y″-chromosome. Neither kind of spermatozoa has a gametic lethal constitution even if present in 50 per cent. of all spermatozoa. This is evident for the X-group. For the Y″-group it follows from the fact that $X\hat{Y}'$ Y″ males form 50 per cent. $X\hat{Y}'$-spermatozoa and 50 per cent. Y″-spermatozoa, all fertile (Y′ is another fragment of a Y-chromosome attached to the X-chromosome).

As the gametic constitution can not account for the behavior of the spermatozoa in Y-deficient males it seemed possible that factors external to the gametes themselves were responsible, such as influences from the surrounding diploid, Y-deficient tissues. In order to test this hypothesis transplantations of testes from genetically fertile into Y-deficient males and *vice versa* were performed.

Similar transplantations were made by Dobzhansky and Beadle (1936) in order to analyze the sterility of hybrid males in crosses between different races of *Drosophila pseudoobscura.* The inability to form spermatozoa in these hybrids is associated with visible abnormalities in diploid germ cells, so that the problem of gametic sterility investigated in our experiments concerns a different aspect of the sterility question.

The method of Ephrussi and Beadle (1936) was used in carrying out the transplantations.

Experimental Procedure

The constitution of the sterile males used was XY″, with "carnation" and "bobbed" in the X-chromosome. They were derived from crosses of homozygous carnation bobbed XX females to "garnet[2] Bar" males of the chromosomal formula $X\hat{Y}'$ Y″. The use of females containing bobbed assured us that no normal Y-chromosome was brought in by an occasional XXY female. The frequency of crossing-over between the *XY′* and Y″-chromosomes in the male was sufficiently low, that no case of reconstitu-

tion of a normal Y-chromosome was discovered during our experiments. The fertile males used as hosts were of the constitution "apricot vermilion" or "white." The testes of apricot vermilion males are slightly colored, those of the white males entirely colorless, while those of the carnation bobbed males are distinctly yellow. These differences in color made it possible to distinguish the implanted testes from those of the hosts. Unexpected modifications of the coloration were observed only in two cases. This problem will not be dealt with in the present report.

A larval testis was transplanted into each larval host. The hosts generally were ready for pupation within one day. Some of the donors were of the same age. However, in order to insure a higher percentage of successful operations, it proved better to transplant the testes from somewhat younger males which have smaller gonads. After the operated males had emerged from the pupa case they were mated to virgin females of the constitution apricot vermilion or white in order to test the transplanted gonad for its power to produce offspring. About five to seven days after having been mated the males were dissected. Both the topographical arrangement of the implanted and of the host testes were noted as well as the motility or immobility of the spermatozoa.

THE DEVELOPMENT OF THE TRANSPLANTS

In 73 individuals the transplantations were successful (Table 1). In 47 cases the transplanted testis in the adult

TABLE 1
SUMMARY OF SUCCESSFUL TRANSPLANTATIONS

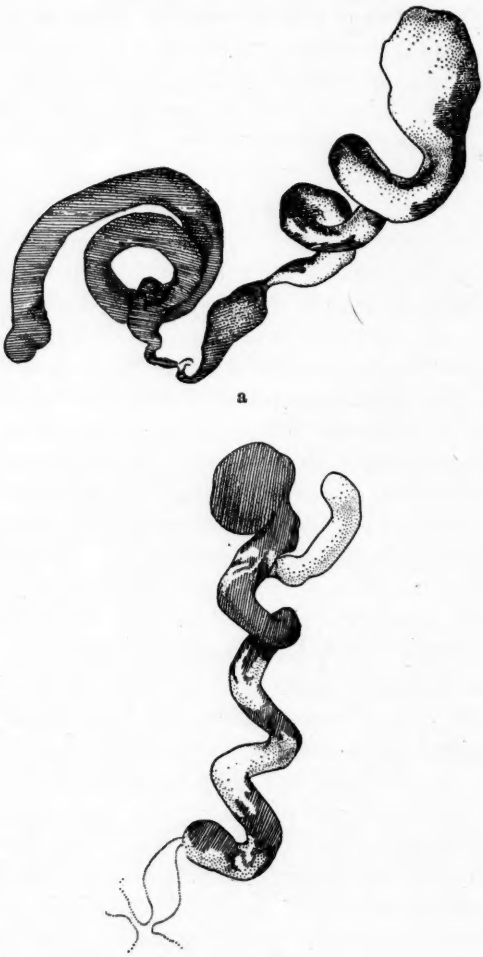
Donor	Host	Transplants			
		Not attached to vas	Attached no compound testis formed	Attached compound testis formed	Attached[1]
Sterile	fertile	33	6	5	–
Fertile	sterile	14	5	8	2

[1] Attachment known to have occurred from progeny test only.

male was found to be not attached to a vas efferens but "free." In the remaining 26 cases a connection with a duct had been established. In this second group an interesting phenomenon was observed, which in a lesser degree had already been encountered by Dobzhansky and Beadle (1936). While in about half of the cases the implant had completely replaced the host testis in being attached to a duct, in the other half the implant and a host testis had grown together. As the coloration of the two genetically different gonadal tissues behaved autonomously to a large degree, it was possible to distinguish the parts of the compound testis which were derived from the host from those derived from the implant. Different degrees and kinds of mixtures were found, such as end to end junction of the two original testes, or longitudinal fusion, or intermingling of tissues forming a complex mosaic (Fig. 1). No case of attachment of two separate testes to one vas efferens nor of a compound, not attached testis occurred.

The mechanism of testis fusion is unknown. Perhaps the normal attraction between gonad and duct, which probably exists, leads often to an attachment of two testes to one vas. In case each of these testes establishes an open communication with the same vas the possibility of fusion seems apparent. It may be mentioned also that when a "free" testis is present it is usually loosely connected by means of tracheae with the distal portion of another testis which is normally attached to a vas efferens.

Testes which have not become attached to a vas retain their larval ellipsoidal shape instead of growing into long spiral tubes (Dobzhansky, 1931). Generally they contain no fully developed spermatozoa. In at least one case, however, we observed normal motility of the spermatozoa of such a free testis. This finding is significant in view of discussions as to the rôle of the paragonial secretion in relation to motility of the spermatozoa.



a

b

FIG. 1. Compound testes. The genetically fertile testis has given rise to the light parts of the compound, while the genetically sterile testis has formed the dark parts.

a. *left*, the sterile host testis with small, empty vas efferens; *right*, the compound testis with larger vas efferens, containing motile sperm.

b. another case of a compound testis.

Fertility and Sterility of Host and Donor Testes

(*A*) *Transplants of sterile testes into fertile hosts:* An analysis of 44 successful experiments (Table 1) gave the following results:

(1) The sterility of the implanted testes was not influenced by their new environment. This is shown by the six implants which became attached to a vas and did not fuse with a host testis. Though completely surrounded by host tissue of normal, not Y-deficient constitution, they remained sterile. Furthermore, the five transplants which had formed compounds with one host testis did not produce fertile spermatozoa, as will be shown below.

None of the 33 "free" transplants contained motile spermatozoa; however, the imperfect development of free testes in most cases does not lead to motility, even when of normal genetic constitution. Thus no motile spermatozoa were found in any of the 11 cases in which one of the host gonads did not become attached to a vas.

(2) Judging by sperm motility, the progeny test or both, the fertility of the host testes when attached to a vas was not impaired by the presence of the sterile implant nor by the operation. This was true for 66 testes in which both original gonads of an individual were attached to a vas, for five testes from males in which the transplant became attached separately, and for five other host testes in which the partner testis had undergone fusion with the transplant. Sterility, probably of an accidental nature, was met with in four host testes (2 males).

(*B*) *Transplants of fertile testes into sterile hosts:* Analysis of 29 successful experiments (Table 1) gave the following results:

(1) The fertility of the implants was not influenced by their new environment. This is shown by four of the five implants which became attached to a vas and did not fuse with a host testis. Though completely surrounded by

host tissue of sterile constitution, they remained fertile (one was sterile). Furthermore, all eight transplants which had formed compounds with one host testis produced fertile spermatozoa, as will be shown below. In two other cases, fertility of a transplanted gonad occurred, as shown by the progeny. However, these males could not be dissected successfully so that it is not known if the implanted gonad had remained separate from a host testis or had formed a compound structure.

Motile spermatozoa were found in one of the 14 free transplants. The sterility of the other 13 testes must be attributed to the imperfect development of a not-attached testis.

(2) The sterility of the host testes persisted in spite of the presence in the same individual of an XY-testis. This was true for all cases (28 gonads) where both host-testes became attached to their vasa; for two gonads, each attached to a vas from males in which the other vas had made connection with the implanted testis; and for seven gonads, each attached to a vas from males in which the implant had fused with the other host testis and the compound had become attached to the other vas.

(*C*) *The compound testes:*

In three cases obtained by combining sterile donor and fertile host, and in eight cases from the combination fertile donor and sterile host, a compound gonad, consisting of one host and one implant testis, was found. In one male from the first combination one host testis had remained unattached, while the other, together with the implant, seemed to have formed two mixed gonads separately attached to the two vasa. In all 13 testes motility of the spermatozoa was observed. Three males with a compound testis did not produce offspring. The progeny of the other nine males (Table 2) showed that only the spermatozoa produced by the gonadal tissue of fertile genetic constitution were able to fertilize. From this it is deduced that the motile spermatozoa observed in com-

TABLE 2

NUMBER OF OFFSPRING FROM THE FERTILE SPERMATOZOA OF MALES WITH COMPOUND TESTES

Donor	Host	Exp.	Apricot vermilion or white	
			F_1 ♀	F_1 ♂
st	f	18	11	5
		40	115	108
		77	69	92
f	st	35	30	23
		61	143	141
		62	59	65
		100	25	8
		103	8	7
		119	36	35
	Total		496	484

pound testes were derived only from the original genetically fertile gonad.

An inspection of the distribution of the two genetically different tissues in compound testes as judged by their coloration demonstrates the fact that each compound gonad had a composition peculiar to it. There were cases where most or all of the fertile tissue occupied the distal end, others with the sterile tissue at this end, still others with each original gonadal tissue forming one longitudinal half of the compound spiral, and finally complicated mosaic compounds. At least in the majority of these cases true mixtures of the spermatozoa from the two original gonads must have existed. In spite of this only the one kind ever reached the fertile stage.

A histological investigation of sections through a few compound gonads showed that the two original testes had indeed formed one common lumen or that the two lumina of testes which had only incompletely grown together opened into one common cavity.

DISCUSSION

The different experiments agree in demonstrating autonomous determination of both host and transplant testes in regard to fertility and sterility. The only apparent discrepancy—the occasional sterility of genetically fertile attached testes and the sterility of most genetically fertile free testes—can easily be seen to be due to acci-

dental causes or to the imperfect development of free testes.

The autonomous behavior of the testes demonstrates that the genetic constitution of diploid gonadal tissue affects the motility of the gametes. Our results permit specifying to some extent at which stage and where this influence may be exerted. Shen had found that the lowermost part of the testis, near to the opening into the vas efferens, possesses a peculiar histological structure. While the testis wall in other parts shows a very thin epithelium, it is here built up of relatively large cells with conspicuous nuclei. An obvious assumption based on these observations was perhaps that the end of the testes is responsible for fertility or sterility; for in this part the spermatozoa become motile in XY-testes and degenerate in Y-deficient testes. If one assumes that the large epithelial cells in normal males furnish secretions necessary for sperm motility one could further assume that the secretions in Y-deficient males were different, bringing about degeneration.

The evidence from compound testes contradicts such assumptions. If the fate of the spermatozoa were determined at this late stage by a general influence of the testicular epithelium, one would expect a uniform behavior of all gametes in a given gonad, regardless of their origin from the host or implant tissue. In case the end portion of a compound testis had been formed by XY-tissue, all spermatozoa should have been motile, while in case this portion was derived from Y-deficient tissue, all spermatozoa should have degenerated. When the end portion itself was a mosaic of both host and donor tissue as in the gonads reproduced in Fig. 1, the assumed secretions should have again affected all gametes alike. But the progeny tests indicate that only gametes from genetically fertile gonadal parts become functional in compound testes. Furthermore, no case of complete inactivity of all spermatozoa has been found. Thus the fertility of

gametes in XY males and the sterility of gametes in XY″-males must be determined earlier. This may occur during spermiogenesis, when the spermatids derived from a single primary spermatogonial cell remain together in bundles and could be jointly subjected to localized influences of the surrounding diploid cells. It seems possible even in compound testes that spermatids from host and transplant are sufficiently separated from each other to account for the localized influences which determine the fate of the spermatozoa. During one stage, rather late in the transformation of spermatids into mature gametes, the former are closely associated with specific somatic cells. Spermatids, in groups of about fifty and probably of common descent, have their heads inserted into a large nutritive cell. While the spermatids transform into spermatozoa, the nutritive cells become smaller and disappear (Guyénot and Naville, 1929). It would seem possible, therefore, that the differential action between an XY- and an XY″ constitution is restricted to effects produced by the nutritive cells.

The interpretation given above that the sterility of Y-deficient males is determined by insufficient or deleterious influences exerted from somatic cells of the testes upon the spermatids is not the only possible one. It is possible that the gametic sterility is already predetermined in the diploid phase of the germ cells. In this case the zygotic constitution would exert an effect on the nongenic content of the future gamete, whether in the nucleus or in the cytoplasm. Which of the two explanations given is valid can not be decided without future work.

Summary

It has been known that males of *Drosophila melanogaster* with an incomplete Y-chromosome produce morphologically normal but immobile and degenerating spermatozoa. This sterility is not due to the genetic constitution of the gametes. It is shown by means of

transplantations of sterile testes into fertile larvae and of fertile testes into sterile larvae that the sterility is either caused by early localized somatic influences on the developing gametes or is an autonomous gametic property due to predetermination in the diploid phase.

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ON GENE STARVATION

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THE gene is a biological unit comparable with those units of the physical sciences, the atom and the molecule. Furthermore, geneticists have developed their concept of the gene by the same kind of reasoning that the chemists and physicists have used to deal with their units. The evidence, also, is strikingly similar, even though it has been gained by biological techniques.

For example, a given gene occupies a position with respect to other neighboring genes which is uniform throughout all the cells of a tissue, just as a certain atom occupies the same position relative to its neighboring atoms throughout all the molecules of a compound. The analogy to chemistry may be continued. The gene can be transferred bodily from one set of neighbors to another (crossing over, translocation, inversion) and is bound to certain neighbors tighter than to others. Also, the chemical principle of combining proportions is involved in the concept of genic balance.

A given gene in association with certain other genes and under certain conditions repeatedly produces the same biological result. When this gene changes or mutates the biological result is usually different in some way or another. The array of different biological results which arise by mutation of a single gene (multiple allelomorphs) shows that there is a limited number of definite stopping places, or stable states, each of which seems to be reached at a characteristic frequency. In addition, the process of mutation is reversible. Mutant genes occasionally return to their original states.

There is such a rigid determinism about the rôle of genes in the development of the organism that most geneticists regard the unmutated gene, as it passes from

one generation to another, as fixed in structure and composition; just as an atom or a molecule which exhibits unchanged physical and chemical properties is regarded as having the same component parts arranged in the same way.

Like living things in general, genes have the capacity of multiplying their numbers. The two daughter genes resulting from the duplication of a single parent gene are identical with it in composition, structure and symmetry. At least, it seems necessary to assume perfect duplication for the genes of the germ cells which connect two identical generations of organisms.

When a gene duplicates itself to produce two daughter genes it seems reasonable to suppose that the materials for one additional gene are collected from the surrounding medium. For the purposes of this discussion it is not necessary to make any assumptions about the structure of the gene nor of the process by which the materials for a new gene are assembled, so long as we suppose that the daughter genes are identical with the parent gene in structure and composition.

There are a number of considerations indicating that the gene has a variety of component parts which are collected in considerable amounts from the medium surrounding the parent gene at the time of duplication. Three or four methods of estimating gene size agree that it has a diameter of the order of 50 millimicrons. Therefore, many thousands or even millions of atoms are involved. Variety of component parts is suggested by the large number of different viable allelomorphs which have been obtained by mutation from certain genes. The latter evidence is especially convincing among allelomorphs which can not be arranged in a single quantitative series. And then, there is the fact that gene sets of both plants and animals regularly show several hundreds or thousands of individual genes, no two of which behave alike.

If we look on the process by which a gene gathers and assembles the materials for an additional gene as nutri-

tion, then the possibility of starvation of the gene naturally suggests itself. We may say a gene has a complete diet when its different components are available in sufficient numbers to build a counterpart. A gene is starved when it can not collect all the parts for another identical gene between two successive divisions of the gene strand. If unmutated genes are fixed in structure and composition, then a daughter gene lacking one or more parts is genetically different and may be expected to produce a phenotypic change in the cells or individuals carrying it.

If a daughter gene is incomplete at the time of chromosome division, then one of three things seems certain to happen. (1) It will go on without the missing part and behave as a mutant allelomorph to the parent gene; or (2) it will substitute something else for the missing part and continue as another mutant allelomorph; or (3) it will disintegrate or fail to divide and constitute a gene deficiency. It should be noted that all three of these possible fates of a starved gene may be expected to produce effects which are already very familiar to geneticists.

Having arrived at this stage in our speculation, we are at once presented with the possibility that gene mutations may be caused by the momentary scarcity of certain gene stuffs in the vicinity of certain genes. Deficiencies in the food of organisms, or other external influences which alter the distribution or availability of certain gene stuffs, may be expected to exert a directive action on mutation and leave the way open for a sort of Lamarckism. This directive action would be expected to restrict the variety of mutations offered for natural selection of the ordinary kind.

Considerable significance may be attached to the fact that eggs do not eat. After an egg has been fertilized it seems to insulate itself from all the disturbing external influences that it can. Then it appears to settle down to the business of performing a series of nuclear and cell divisions, during the course of which most of the tissues and organs of the adult organism are differentiated and

started on their way. As soon as this differentiation is accomplished, the developing organism adopts an "open-door policy" and begins to carry on an active commerce with the outside world. It now takes in a miscellany of food substances, of which some are used in the growth of the differentiated tissues, some are burned for their energy and the rest are stored or voided.

When an egg is examined it may be seen that its contents are different in different regions. Certain areas, segments or layers are colored differently from others. Some have granules and some do not. Some places are opaque and some are hyaline. This pattern of cytoplasmic differentiation is remarkably uniform among the eggs of a single species. It appears that these various substances have been sifted and sorted and segregated with precision.

If the different substances which enter into the makeup of the new gene sets (built during the period of proliferation) are also unequally distributed, or if some of the different substances which we see are used in making genes, then it follows immediately that genes in different regions of the cytoplasm will undergo different kinds of starvation. If the mosaic of gene-forming stuffs is the same throughout the eggs of a species, then the pattern of starvation will be equally uniform. According to the conclusions reached earlier in this discussion, these different kinds of starvation will result in different gene mutations or deficiencies among the nuclei located in different parts of the cytoplasm. These genetically-altered nuclei, and the cells which inclose them, may be considered the primordia of the various tissues and organs. The characteristics by which we distinguish differentiated tissues and organs will then be the phenotypic expressions of these gene changes.

A number of zoologists have, from time to time, summarized the evidence of embryology and genetics. Quite commonly they have emphasized the difficulty and lack of

success in harmonizing the results of these two techniques. I suspect that the construction of a general theory may have been delayed by a statement which has persisted for a great many years. This statement is that all the cells of the body receive unaltered the same sets of genes as were present in the fertilized egg. I believe most geneticists will agree that the evidence which shows that the gene sets reproduce themselves unaltered pertains to phenomena taking place in the cells of the germ track. This evidence is lacking for the genes of the somatic cells even though they show a number of points of similarity to those of germ cells. There is rather direct evidence, derived mostly from tissue culture and transplantation experiments, which goes to show that differentiated tissues maintain their differences just as certainly as genetically different organisms living in the same environment and nourished by the same foods maintain their differences. There are also persisting effects of temporary starvation and diet deficiencies on organisms which may be regarded as aspects of differentiation. It seems to me that there is a fair likelihood that experiments designed to test the effects of starvation on genes may play an important part in the formulation of a theory which will fit the facts of both embryology and genetics.

Throughout this discussion it has been implied that all gene changes resulting from starvation have been toward simplicity and uniformity of composition among the genes of a set. This is because all those genes or gene parts would be expected to be eliminated (at the time of division) except those whose materials are available in the milieu of the gene set. It would appear that these gene changes resulting from starvation are predominantly irreversible because the bulk of the evidence shows that once cells become differentiated they stay differentiated. Recent workers seem inclined to give other explanations to those tissue changes that were formerly regarded as instances of dedifferentiation.

It is necessary to assume that the totipotent germ cells need to have their genes preferentially nourished in order to reproduce their own kind—like queen bee grubs in a hive. It can be supposed that the germ cells proliferate in certain regions of the cytoplasm where all the gene stuffs are present. Successive differentiations in the same tissue may be accounted for by a sequence of different gene starvations. It is conceivable that undifferentiated cells may have their genes starved for one substance to form epithelium. Later, some of these epithelial cells may have their genes starved for another substance to produce nerve cells.

In those cases where blastomeres isolated from a developing egg can form a complete individual, an explanation in terms of gene starvation does not come so readily. One must not only assume that the gene-forming stuffs become segregated after cleavage has begun, but also that this segregation is controlled by something associated with isolation—perhaps the distribution of oxygen tension over the surface of the blastomere. The same explanation could be used for those instances where more than one egg enters into the formation of a single individual. Regeneration, polyembryony, etc., seem too complicated to discuss profitably from this viewpoint at this time.

OBSERVATIONS ON THE OSSIFICATION OF THE FOOT-BONES IN POLYDACTYL AND NORMAL CHICKS[1, 2]

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INTRODUCTION

POLYDACTYLISM in the chick has been the subject of numerous genetic investigations. The anatomy of the condition has been outlined in these papers. Very little work has been done on the embryology of the polydactyl foot. Research on the development of the condition, besides being of interest to the embryologist, may aid in the interpretation of some of the genetic relationships of polydactylism. The purpose of the present investigation has been to give an outline of the ossification processes occurring during embryonic life. Incidental observations on normal development have also been recorded.

REVIEW OF LITERATURE

The early development of both the normal and polydactyl foot of the chick has been outlined by Kaufmann-Wolf (1908). Her material included embryos of five to nine days' incubation age.

The early development of the pelvic bones and the tarsal elements of the normal chick has been given by Johnson (1883). She gives references to Rosenberg (1873), who first observed the "fifth metatarsal" and to Morse (1880) and Baur (1883), who attempted to determine the homologies existing in the tarsal region.

The time and order of ossification of the normal chick foot has been determined by Strong (1920). Similar investigations have been made on the human embryo by

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[2] The authors wish to express their gratitude to Dr. D. C. Warren, of the Department of Poultry Husbandry, for his cooperation in furnishing the material for this investigation and for reading and criticising the manuscript.

Mall (1906), on the rat by Spark and Dawson (1928) and Strong (1925), and on the fore leg of the guinea-pig by Harman and Saffry (1934).

Observations on the influence of the sex factor on bone development have been made by Pryor (1925) and Harshaw (1934) as well as by Spark and Dawson (1928) and Harman and Saffry (1934).

From experimental observations on chick blastoderms Rawles (1936) presents evidence which indicates that the left side of the chick is developmentally superior to the right side. Bilateral variation in polydactylism has been emphasized by Bond (1920) and Fisher (1935). These two authors and others have attempted to systematize the knowledge of the anatomy of the polydactyl condition. A method of clearing and staining preparations for the demonstration of bone deposition has been described by Dawson (1927).

MATERIAL AND METHODS

Material for this work on chick embryology was furnished through the courtesy of Dr. D. C. Warren, of the Department of Poultry Husbandry at Kansas State College. A total of 149 embryos was examined. Of these, 58 were normal and 91 were polydactyl. A number of feet of hatched chickens of various ages, both polydactyl and normal, were also examined. The embryos were fixed in 95 per cent. alcohol. Their feet were removed, cleared in potassium hydroxide solution, and stained with alizarin, according to the method described by Dawson (1927). Both feet of each embryo were prepared. Feet of hatched and adult birds were studied both as dissections and as cleared and stained preparations. The smaller specimens were examined by means of a dissecting microscope.

The embryos examined ranged from seven to nineteen days' incubation age. The sex of the embryos was determined by dissection.

Thirty-six normal embryos, aged from 10 to 19 days, were considered old enough that determination of their

sex by dissection was fairly certain. Of these, 15 were female, 19 male and one doubtful. Of 59 polydactyl embryos aged from 10 to 19 days, 26 were male, 27 were female, two were doubtfully identified as female, two as male and the sex of one was wholly doubtful.

In this paper, the word polydactyl is used in referring to all birds of polydactyl stock, whether the doubling of the hallux is evident or not.

Observations

The ossification processes of the normal foot were studied in order to furnish a basis for comparable work on the polydactyl foot.

Ossification in the Normal Foot: Ossification in the leg begins at about the end of the seventh day of incubation. Ossification centers for the tibia, fibula and femur are present at seven and one-half days. Three separate complete centers for metatarsals II, III and IV have appeared by the end of the eighth day. No more ossification centers appear until the eleventh day, when the phalanges begin to ossify. An ossification center appears in metatarsal I late on the twelfth day.

By the fifteenth day all 14 phalanges have begun to ossify. The single exception to this statement was found on the right foot of one bird, in which there was no center present for the fourth phalanx of the fourth toe. This phalanx is the last to begin ossification in all cases examined. It is also the shortest of all the phalanges.

A separate tarsal element, the "ascending process of the astragalus" begins to ossify during the latter half of the fourteenth day. No other tarsal ossifications begin until the eighteenth and nineteenth days, when ossification centers appear for a fibulare, a tibiale and a single central element.

The time and order of appearance of the ossification centers of the normal chick foot are shown in Table I.

The time at which a center appears may vary as much as a whole day. The order of ossification is constant as

TABLE I

TIME AND ORDER OF APPEARANCE OF OSSIFICATION CENTERS IN THE NORMAL CHICK FOOT

Incubation Age—Days	Digits				Tarsalia
	IV	III	I	II	
8	metatarsal	metatarsal		metatarsal	
9					
10					
11		1		1, 2	
			1		
		2			
		3			
	1				
12		4			
	4		metatarsal	3	
13			2		
	5				
	2				
13½					Ascending process of the astragalus
14					
15	3				
16					
17					
18					fibulare tibiale Central ossification

Roman numerals represent the first, second, third and fourth digits. Arabic numerals under each digit number represent phalanges in that digit, beginning with number 1, the basal phalanx, and continuing distally. The order of ossification for each digit may be read on the column under the digit number. The order of ossification in the entire foot may be read vertically, passing from column to column.

far as it was determined for all the specimens studied. In Table I only the earliest time of observation for each center is recorded. In this way the table is made to show the order of ossification unobscured by the variations in the time of appearance of the centers.

One bird did not conform to the order of ossification as shown above. In this embryo, aged 12 days, the left foot alone was completely available for examination. No center for the first metatarsal appeared on this specimen, but a center for the second phalanx of toe I was present. In all other chicks this center does not appear until after metatarsal I has begun to ossify.

The patella was not ossified in any of the embryos examined. Ossification in the long bones of the foot begins

perichondrally. A thin ring of bone is formed around the middle of the diaphysis. During the early stages of ossification of the second phalanx of toe IV, two separate ossification centers, one lateral and one medial, were seen. A day later these two centers were fused to form a complete ring. The basal phalanx of the hallux begins ossifications as a dorsal saddle-shaped center.

The terminal phalanges (claws) ossify from separate hook-shaped dorsal and plantar centers, which soon fuse proximally but remain separate distally for some time. The plantar center appears before the dorsal one. Traces of this double origin may be observed even in the claw bone of the adult bird.

Ossification of the first metatarsal begins proximally and generally appears in the form of a cap over the tip of the cartilaginous metatarsal I. However, the center may appear as a ring near the proximal tip of the cartilage.

No separate epiphysial centers of ossification were observed on any of the embryos examined. We are not considering as epiphysial ossifications the separate tarsal bones formed during embryonic life, although they later fuse with the ends of the tibia and the cannon-bone. The diaphysial ossifications of the long bones are tubular and open at the ends in the oldest embryos studied. Especially in the younger stages of ossification the diaphysial centers of the long bones are noticeably thinner at the ends than they are at the middle.

Ossification of the "ascending process of the astragalus" begins at the proximal tip of its heart-shaped cartilage and proceeds distally. The lateral and medial tarsal elements (fibulare and tibiale) begin as disk-shaped or saucer-shaped centers. (Fig. 2, FI and TI.) The single element between these and distal to them appears later as an irregular, rather diffuse ringlike center.

Metatarsals II, III and IV come in contact with each other during the twelfth day. Metatarsals II and III touch each other before metatarsal III touches IV. The point of the first contact is somewhat distal to the middle

of the diaphysis. Fusion does not take place until about the sixteenth day. The ends of these three metatarsals diverge slightly and remain separate for some time after hatching. They are separated distally even in adult life.

During embryonic life and for some time thereafter, the four tarsal ossifications remain separate from each other and from the cannon-bone and tibia.

A few slight bilateral variations in time and extent of ossification were observed. They were not consistently in favor of either side.

The Fifth Toe: Although the technique employed in this investigation was one designed primarily to show ossification, it happened that occasionally a fortunate preparation showed cartilage as well. Because of this, we were able to observe some of the pre-osseous stages in the skeletal development of the foot.

On the outer or fibular side of the tarsal region in feet of embryos aged seven and nine days, a small separate cartilaginous nodule was observed which in position and appearance corresponds to the "fifth metatarsal" as described by Rosenberg (1873), Baur (1882), Johnson (1883) and others. We have not observed its fate. Fig. 1 shows the fifth toe as it appears in the foot of a nine-day chick embryo.

THE POLYDACTYL FOOT

Anatomy: In all but two of the polydactyl birds which we examined, the double condition, when it did appear, was confined to the region of the hallux. In the two exceptions, which were embryos aged 10 and 11 days, the third and second toes respectively appeared split at the tip. There was no ossification present, and we are unable to describe them further.

The polydactyl condition may be manifested as a single hallux with four instead of three bones (a four-toed condition); two halluces may be present, either separated or in varying degrees of union (five-toed foot); and either of these toes may also appear split distally (six-toed

foot). No case of splitting of both toes on the same foot (seven-toed condition) is known to us. Either of the halluces on the five-toed foot may contain more or fewer than the typical number of bones in the normal hallux. Fig. 3 is a polydactyl foot of the four-toed type; Fig. 4 is a foot of the five-toed type.

Generally, separation of two divisions of the hallux is more evident terminally than basally. Only one or two specimens which exhibit proximal separation are known to us, and here it is possible that the slight distal fusion was secondary.

Either one of the two primary divisions of the hallux may appear as a single dwarfed phalanx or even as a metatarsal alone.

Forked or Y-shaped bones are often found in specimens in which the parts of the hallux are not completely separated. The branches of the Y may be equal or unequal in length, and the cleft, which is almost always distal, may be shallow or deep.

The number and size of phalanges in toes II, III and IV is in general the same as in the normal foot; but the phalanges in the region of the hallux vary extremely in size, shape and arrangement.

The first metatarsal on the polydactyl foot may be in one or two pieces. If in one piece, it may be extremely large and long, lying diagonally across the cannon-bone, possibly in a groove in it. The head of metatarsal I may lie in a socket on the inner side of metatarsal II (Fig. 4). A large first metatarsal is not always associated with an extreme condition of polydactylism. When two metatarsals are present, the outer one may lie in an abducted position, so that it looks almost like a phalanx. There is usually, however, little doubt as to whether such a bone is a metatarsal or a phalanx.

The two feet of one bird often show different types of polydactylism. In a considerable proportion of birds the polydactyl condition is not evident at all. It should be observed here that most of the so-called polydactyl stock

used in this work resulted from matings of heterozygotes which should give approximately one fourth normal offspring.

Of 23 polydactyl embryos in which the number of bones in the hallux was evident, seven showed no doubling of either hallux, and four showed the same extent of doubling of both halluces. Of the 12 birds exhibiting bilateral variation in degree of polydactylism, five showed more doubling on the right than on the left. The phalanges of the right hallux were either more numerous or better developed than those of the left hallux. Seven heterodactylous embryos showed more doubling on the left than on the right. An apparently normal foot was combined with a polydactyl foot on only one of these birds. In this case the normal hallux was on the right side. The above observations are based largely on the study of older embryos, but we have not seen any adult condition which conflicts with them.

Ossification in the Polydactyl Foot: In the polydactyl feet there is wide variation in the time of appearance of ossification centers. The following are some of the more outstanding examples of this variation:

Polydactyl Feet:

(1) An ossification center for the third phalanx of toe IV appears as early as the fifteenth day, yet this center is absent in one 17-day embryo.

(2) An ossification center for the ascending process of the astragalus appears as early as 13½ days, yet a center for it was absent on one 17-day embryo (the same 17-day chick as in I).

(3) An ossification center for metatarsal I appears as early as nine days, yet a center for it is absent in several 13-day embryos.

Normal Feet:

(1) An ossification center for the third phalanx of toe IV appears on all 15-day feet; the center for it is absent on the right foot of one 16-day embryo.

(2) An ossification center for the ascending process of the astragalus appears on one 13½-day bird, is absent on one of two 14-day birds, and is present thereafter.

(3) An ossification center for metatarsal I appears on half of the 12-day feet examined, and is present on all feet of birds aged 13 days or over.

Table II gives the time and the order of appearance of the ossification centers in the polydactyl foot. The earli-

est appearances are the ones recorded. For the hallux, the earliest appearances of the first metatarsal and the phalangeal ossifications for both polydactyl and apparently normal feet are recorded.

TABLE II

TABLE SHOWING TIME AND ORDER OF APPEARANCE OF OSSIFICATION CENTERS IN THE POLYDACTYL CHICK FOOT

Incubation Age—Days	Digits					Tarsalia
	IV	III	I		II	
			Double	Single		
7½	metatarsal	metatarsal				
8					metatarsal	
9			metatarsal			
10					1	
11		1				
		2, 3		1	2	
	1					
12		4	1 (first phalangeal center.)			
13				2 metatarsal		
	5					
	4					
13½						ascending process of the astragalus.
14						
15	2					
	3					
16						
17						
18						
19						fibulare tibiale centrale

The phalanges as well as the metatarsal of the hallux in the polydactyl foot show variation in the time and order of their appearance. In polydactyl feet without a double hallux, ossification centers for phalanges of the hallux were observed at 11 days. Such centers were not observed until 12 days in feet showing the polydactyl condition. In the upper division of the hallux ossification may or may not proceed proximo-distally.

Tarsal ossifications in the polydactyl foot usually appear later than they do in the normal foot. (Compare Figs. 2 and 3.)

No constant order was observed in the appearance of centers either in the upper or lower division of the hallux in feet showing the polydactyl condition.

Normal phalanges and metatarsals in the polydactyl foot begin ossification in the same manner as do corresponding bones in the normal foot. Separate metatarsals in the region of the hallux begin as separate caplike ossification centers.

A forked phalanx may ossify from one ringlike center or from two. Ossification begins at the middle of the diaphysis, and if the cleft extends past the middle, two centers are formed; if the cleft is shallow, only one center is formed.

Bilateral variation was marked in the region of the hallux. Since the type of polydactylism also varied bilaterally in many cases, variation aside from this was not distinguishable with certainty.

The Fifth Toe: The cartilaginous representative of a fifth toe was observed on several of the younger polydactyl embryos, aged seven and nine days.

Miscellaneous Observations: At least two polydactyl embryos, aged 13 and 17 days, showed exaggerated endochondral ossification in the bones of the foot. In bones showing this condition a rod or core of alizarin-staining material extended through the middle of the ring-like perichondral center and projected for some distance at each end. Both embryos were male.

Twelve polydactyl embryos, including the two abnormal ones just described, were found in which metatarsals II, III and IV were bent sharply backward near their proximal ends. In two birds the tibia was also bent backward near its distal end. Doubling or lengthening of the hallux was evident on only four of these birds. The bending of the metatarsals appears to be associated with their unusually early fusion, and in some cases with fusion

between them and metatarsal I. Six of the embryos showing this condition were 13 and 13½ days old. Four were younger (9, 11 and 12 days), and one (17 days) was older. Eight of these embryos were male and four were female.

Nine polydactyl embryos were behind others of their age in ossification (lacking two or more centers as compared with the average polydactyl bird of the same age). Six of these were male, two female, and one was doubtfully identified as female. Because of variation in the hallux, ossification centers in this toe were not considered here.

Six polydactyl birds were more advanced in extent of ossification than others of their age. Three of them were male and three were female. None had bent metatarsals.

Of four normal birds behind others of their age by at least two centers, all were male. Few if any normal birds could be classed as definitely ahead of others of the same age.

The one polydactyl embryo which did not conform to the order of ossification as generally observed was male. It had bent metatarsals, warty skin, and was found dead in the shell. It was the only embryo so found that was prepared.

Three female polydactyl birds had only four phalanges in the fourth toe—a condition known as brachydactylism. No bones were completely ossified in any of the embryos examined.

Discussion

The main ossification centers in the normal chick foot are formed during the latter two thirds of embryonic life. In general they appear in a definite order. This order is not the same as the order of chondrification as determined by Kaufmann-Wolf (1908).

In the individual toe the order of chondrification is proximo-distal. The order of ossification is not proximo-

TABLE III

TABLE SHOWING ORDER OF CHONDRIFICATION IN THE FOOT OF THE NORMAL CHICK EMBRYO. ARRANGED FROM DATA OF KAUFMANN-WOLF (1908)

Incubation Age—Days	Digits					Tarsalia
	IV	III	I	II	V	
5	metatarsal	metatarsal				fibulare
				metatarsal		central element
					metatarsal	tibiale
	1	1				
6			metatarsal			
7	2		1			
		2				
				1		
8	3					
		3				
				2		
9			2			
	4					
		4				
				3		
	5					

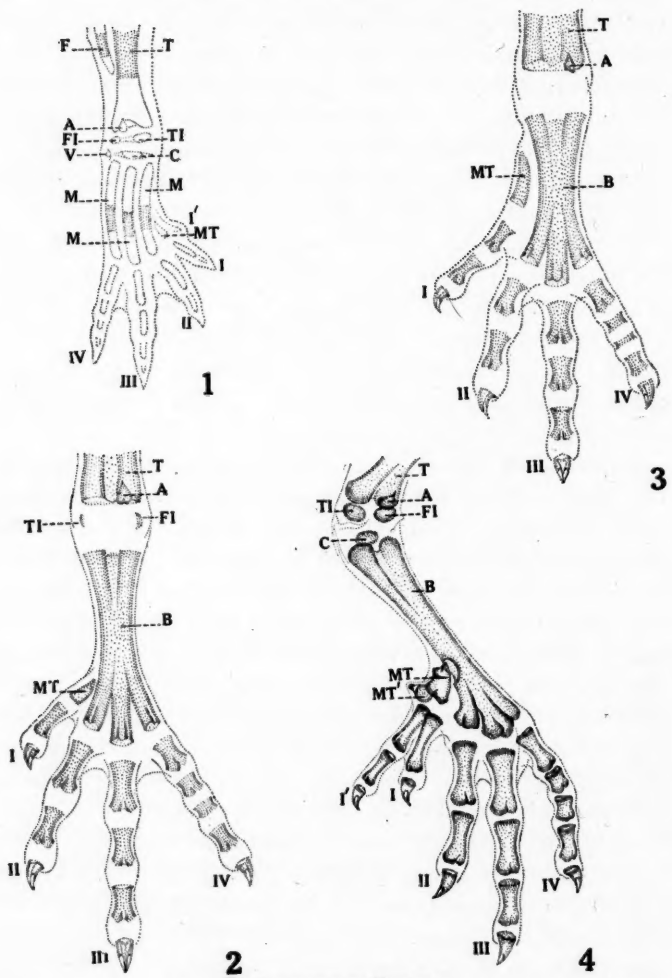
distal in the first and fourth toes. We have made a few measurements of the bones of older feet which indicate that in the individual toe the order of ossification approximates the order of length of the phalanges. The longer bones ossify earlier, even though their cartilage fundament is formed later than that of some smaller bones.

In its skeletal development the chick foot presents a number of points of contrast with the development of the limb in mammals. No independent epiphysial centers of ossification are formed during embryonic life up to 19 days in the chick foot. Such centers are conspicuous in the hind limb of the guinea-pig embryo. In man, according to Mall (1906) and in the white rat, as investigated by Strong (1925) and Spark and Dawson (1928), the terminal phalanges are the first bones to ossify in the digits. In none of the digits of the normal chick is this the case. Harman and Saffry (1934) state that in the guinea-pig "the first phalanges are the first to show ossification centers and the second phalanges are the last." Here again the terminal phalanges precede in ossification

phalanges which are proximal to them. A point to be considered in this connection is the presence in the chick of four digits with different numbers of phalanges. We can not consider the phalanges of all the toes as arranged in proximal, middle and distal ranks. In the guinea-pig, according to Harman and Saffry (1934) the tips of the terminal phalanges arise from two ossification centers, one lateral and one medial. The terminal phalanges of the chick ossify from two centers which are dorsal and plantar in position. No sesamoid bones ossify in the foot of the chick during its embryonic life up to 19 days. Here again the chick differs from the guinea-pig in which animal sesamoid bones are conspicuous during the latter part of fetal life.

Independent tarsal bones, characteristically absent in the adult fowl, appear as separate ossifications during embryonic life. Four centers were observed to arise in the tarsal region before hatching. Several attempts have been made to homologize these centers with the independent tarsal elements of other animals. It is generally agreed that the lateral and medial centers represent a fibulare and a tibiale. The central element has been variously identified with a centrale (Morse) and with the fused distal tarsalia (Johnson). In the ossification of this element we have seen no evidence that it is composed of more than one center. The small pointed element on the front of the fibular side of the tibia (ascending process of the astragalus) has been called the intermedium (Morse) and an outgrowth of the tibiale (Baur, Johnson). The fact that it begins ossification much earlier than does any other tarsal element appears to argue in favor of the theory of its independent origin. The fibulare generally ossifies a little in advance of the tibiale. The postnatal history of these centers and the influence of breed and sex on their time of fusion has been discussed by Harshaw (1934).

According to Kaufmann-Wolf (1908) the extra toe in the polydactyl hallux arises as a cartilaginous outgrowth



EXPLANATION OF FIGURES

FIG. 1 was drawn 13 times natural size: Figs. 2, 3 and 4 were drawn four times natural size. All figures are reduced by one half.

FIG. 1. Right foot of a nine-day polydactyl embryo. The stippled areas represent ossification; the areas outlined with dots represent cartilage. A, ascending process of the astragalus; C, central element of the tarsus; F, fibula; FI, fibulare; M, metatarsals II, III and IV; MT, first metatarsal; T, tibia; TI, tibiale; I–V, digits one to five; I′, the extra toe.

FIG. 2. Left foot of an 18-day normal embryo. The stippled areas represent ossification. Cartilage is not indicated. A, ascending process of the

from the metatarsal or one of the phalanges of the true hallux. The extra toe may or may not separate completely from its "matrix." According to this authority the phalanges of the extra toe characteristically retain cartilaginous connection with each other. Separate centers of ossification, however, arise in the extra toe in much the same manner as they appear in the true hallux. The persistence of normal processes under abnormal conditions is strikingly demonstrated here.

In the polydactyl foot the first metatarsal varies extremely in time of appearance. Whether or not this variation is correlated with the variation in size of the first metatarsal is difficult to say. In any case, the first metatarsal is directly concerned in variations connected with the polydactyl condition. (Compare metatarsals in Fig. 2 and in Figs. 3 and 4.) The other differences between the polydactyl and normal feet may be explained independently of this, perhaps in terms of breed or size difference. In this connection it may be observed that the one disturbance of ossification order in the normal foot was associated with a variation in the time of appearance of the first metatarsal.

Polydactylism in some animals, such as the horse, can be explained as the persistence of a digit normally reduced or absent. Attempts have been made to explain polydactylism in the chick in these terms.

Our observation of a cartilaginous representative of a fifth toe in embryos showing also extra toes confirms observations made by Kaufmann-Wolf (1908), from which we draw the conclusion that the extra toe is not a reappearance of a fifth digit, but rather an indepen-

astragalus; B, cannon-bone (fused metatarsals II, III and IV); FI, fibulare; MT, first metatarsal; T, tibia; TI, tibiale; I–IV, digits one to four.

FIG. 3. Left foot of an 18-day polydactyl embryo. A, ascending process of the astragalus; B, cannon-bone; MT, first metatarsal; T, tibia; I–IV, digits one to four.

FIG. 4. Left foot of a six-weeks postnatal chicken. A, ascending process of the astragalus; B, cannon-bone; C, central element of the tarsus; FI, fibulare; MT, first metatarsal; MT′, metatarsal of the extra toe; T, tibia; TI, tibiale; I–IV, digits one to four; I′, the extra toe.

dent outgrowth from the region of the hallux. Kaufmann-Wolf states that the "fifth metatarsal" later fuses with the cannon-bone and disappears into it.

The first toe, as well as the fifth toe, is rudimentary in the chick. The fifth toe, however, is represented by a proximal rudiment, while the first toe is represented by a distal rudiment, the first metatarsal and its phalanges.

Aside from the irregularity introduced by the development of extra toes, variations in time of ossification are much greater in the polydactyl foot than in the normal foot. Certain authors, including Pryor (1928) and Spark and Dawson (1928) have investigated the possible influence of the sex factor on the ossification processes. We find that there is a tendency toward slower development in the male than in the female. In this investigation we consider the presence or absence of ossification centers, rather than the extent of ossification already present, as indicative of difference. The male birds, more than the females, also appeared to be subject to the disturbing influence of the factor causing the condition of bent metatarsals. These statements must be accepted with the limitations imposed by the small amount of material investigated.

The matter of bilateral variation has received some attention, in the development of both the polydactyl and the normal chick. We have sought to determine whether the ossification processes demonstrate any developmental superiority of one side over the other, aside from the bilateral variations of the actual polydactyl condition, which have been discussed in connection with the genetics of the condition by Bond (1920) and Fisher (1935), among others. Rawles (1936) working with early chick blastoderms, demonstrated that the left side is superior developmentally to the right side. We found a small number of slight bilateral variations, but they were not consistently in favor of either side. Here again we used presence or absence of an ossification center, rather than extent of ossification, as indicative of difference.

Summary

(1) The ossification processes in the normal and polydactyl chick have been studied, and the order and time of appearance of the ossification centers have been recorded.

(2) Ossification begins in the chick foot at about the end of the seventh day of incubation. By the end of the nineteenth day centers are present for all the long bones of the foot and for the tarsalia.

(3) No sesamoid bones ossify in the foot during the first 19 days of incubation. No true epiphysial ossification centers appear during this period. No ossification of the patella was observed.

(4) There appears to be some relation between the size of a bone and the time when it begins ossification. The order of chondrification is not the same as that of ossification.

(5) No consistent bilateral variations in time of ossification were observed.

(6) Ossification begins earlier in female birds than in male birds. More males than females were found abnormal.

(7) Abnormalities observed included two cases of extensive endochondral ossification and 12 cases of bent metatarsals.

(8) Brachydactylism was observed on three female birds.

(9) In its ossification processes the normal chick embryo differs from the rat in the comparatively early beginning of ossification; from the guinea-pig in the lack of centers for sesamoid bones and for true epiphysial ossifications; and from the rat, guinea-pig and man in the order of ossification of the phalanges.

(10) The presence of a cartilaginous fifth digit in polydactyl embryos indicates that the extra toe in these birds is not an over-developed fifth toe. The fifth digit appears on the fibular side of the foot, while the extra toe appears on the tibial side.

(11) Polydactylism in the chick is a condition of hyperphalangy of the hallux, manifested either as a lengthening or as a splitting of the digit. No definite order of ossification was observed in the phalanges of the polydactyl hallux.

(12) It is possible that some birds identified as normal in genetic studies of polydactyly may actually be polydactyl birds manifesting the condition in one of its more inconspicuous forms, such as a lengthened single hallux or a small extra metatarsal.

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THE RELATIVE NUMBERS OF SPECIES OF MARINE FISH ON THE LOUISIANA COAST[1]

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INTRODUCTION

THE data on which this paper is based consist of records of the numbers of fish caught in otter trawls along the Louisiana coast by members of the Shrimp Investigations, U. S. Bureau of Fisheries. The fish were taken in an ordinary commercial shrimp trawl measuring thirty-five feet in wingspread and having a mesh three fourths of an inch square.

Trawling was done chiefly in Barataria Bay and the adjoining waters of the Gulf of Mexico, but occasional hauls were also made in Lake Pontchartrain, Lake Borgne, Mississippi Sound, Chandeleur Sound, East and West Bays at the mouth of the Mississippi, Little Lake and Timbalier Bay. Four hundred and twenty-two hauls were made with fair regularity from July, 1931, to June, 1934, and over 144,000 fish were taken. The localities visited ranged from the landward or inside limit of brackish water to six miles offshore in the open Gulf.

Ecologists have become aware in recent years that a working knowledge of animal communities will have to be based on population studies. Still better and much more difficult would be quantitative studies of the protoplasm involved. Nothing approximating a complete census of fish in a large area is possible, so we have to fall back to a relative or comparative study of species numbers for whatever knowledge of the population it will give.

Any one means of collecting for the determination of relative abundance will have some weakness and the tool used or rather the data gathered by it should be carefully evaluated. Beebe's (1934) observations from the bathysphere indicated to him that trawls are not efficient in the

[1] Published by permission of the U. S. Commissioner of Fisheries.

clear waters of Bermuda. This is to be expected, for fish can dodge a trawl if it is seen in time. Nevertheless, the trawl has been used for many years in commercial fishing, and its use seems to be on the increase. Its observed efficiency in catching marine animals has aroused popular opposition in some instances. Much work has been done on savings-trawls and it appears that special pains must be taken to prevent the trawl from taking large numbers of small fish, once it has been put in operation. The chief objection to the shrimp trawl as an unbiased collector is the fact that it captures more small fish than large ones. This is more fortunate for the present purpose than the reverse situation would be, for the basic or food animals of a community are more numerous and of smaller size, but have more protoplasm in their species than the larger animals, their predators.

The trawl seems to be very efficient in capturing the smaller, more numerous fishes in the turbid, littoral waters of the Louisiana coast. Large fish, those that remain close to shore and the blenny and goby society of oyster reefs are not much affected. These facts will have to be held in mind in evaluating the data given hereafter.

DISCUSSION OF DATA

Table I shows the numbers of each species taken, in decreasing order. Separate figures for the bays and Gulf as well as the totals are given. The average number of each species caught per haul is also given. Two members of the family *Sciaenidae* head the list. The croaker, *Micropogon undulatus,* was caught almost as often as all other fish put together. Almost every place that a trawl can be put overboard in these waters one or more croakers will be taken. The star-drum, *Stellifer lanceolatus,* was second in abundance, followed by the anchovy, *Anchoviella epsetvs.* The anchovy is small and due to its shape easily passes through the trawl meshes. Thousands are shaken out when the trawl is brought aboard. Although it only stands third on the list, it is possible that this

TABLE I

NUMBERS OF FISH TAKEN IN 422 TRAWL HAULS ON THE LOUISIANA COAST. FIGURES FOR INSIDE (BAYS), OUTSIDE (GULF) AND TOTALS ARE GIVEN WITH THE AVERAGE PER HAUL FOR EACH SPECIES

	Total	Average	Inside	Average	Outside	Average
Micropogon undulatus	71,774	170.1	36,573	151.8	34,991	193.3
Stellifer lanceolatus	11,857	28.1	1,783	7.4	10,074	55.7
Anchoviella epsetus	10,961	26.0	7,921	32.9	3,040	16.8
Cynoscion arenarius	10,388	24.6	4,797	19.9	5,591	30.9
Brevoortia patronus	5,944	14.1	5,371	22.3	573	3.2
Galeichthys felis	5,527	13.1	3,845	16.0	1,682	9.3
Leiostomus xanthurus	3,772	8.9	1,709	7.1	2,063	11.4
Vomer setapinnis	3,500	8.3	1,217	5.1	2,283	12.6
Polynemus octonemus	3,219	7.6	2,227	9.2	992	5.5
Trinectes maculatus	2,472	5.9	1,658	6.9	814	4.5
Bagre marinus	1,707	4.0	1,249	5.1	458	2.5
Cynoscion nothus	1,626	3.9	36	0.1	1,590	8.8
Trichiurus lepturus	1,510	3.6	1,335	5.5	175	0.9
Bairdiella chrysura	1,408	3.3	1,225	5.1	183	1.0
Menticirrhus americanus ...	1,392	3.3	186	0.8	1,206	6.7
Poronotus triacanthus	1,362	3.2	510	2.1	852	4.7
Etropus crossotus	1,315	3.1	921	3.8	394	2.2
Citharichthys spilopterus ...	990	2.3	776	3.2	214	1.2
Cynoscion nebulosus	926	2.2	907	3.8	19	0.1
Prionotus (several)	499	1.2	235	0.9	264	1.5
Achirus lineatus	468	1.1	383	1.6	85	0.5
Symphurus plagiusa	452	1.1	242	1.0	210	1.2
Signalosa atchafalayae	309	0.7	242	1.0	67	0.3
Spheroides spengleri	272	0.6	224	0.9	48	0.3
Paralichthys lethostigmus ..	262	0.6	229	0.9	33	0.2
Chaetodipterus faber	257	0.6	85	0.4	172	0.9
Larimus fasciatus	215	0.5	7	0.03	208	1.1

plankton feeder is the most numerous, as well as the largest from the standpoint of species mass, of any fish in littoral Louisiana waters.

Fishes taken in small numbers are not included. Records for these covering part of the period have been given before (Gunter, 1935). The list was arbitrarily ended with *Larimus fasciatus,* a much less common member of the family Sciaenidae than its relative, *Micropogon undulatus.* The table is self-explanatory, and a discussion of each species is unnecessary. Fish that were found to be twice as abundant in the Gulf as in the bays or *vice versa* were arbitrarily said to be more common in the one locality than in the other. The list in Table Ia is based on that procedure and is derived from Table I.

This shows simply from our experience what fish were found to inhabit predominantly the estuarine portion of this area or the open sea. A closer examination of Table I will show that there are all gradations between the two classes from *Cynoscion nothus* and *Larimus fasciatus,*

TABLE Ia

BAYS	GULF
Anchoviella epsetus	Stellifer lanceolatus
Brevoortia patronus	Vomer setapinnis
Galeichtys felis	Cynoscion nothus
Bagre marinus	Menticirrhus americanus
Trichiurus lepturus	Poronotus triacanthus
Bairdiella chrysura	Chaetodipterus faber
Citharichthys spilopterus	Larimus fasciatus
Cynoscion nebulosus	
Achirus lineatus	
Signalosa atchafalayae	
Spheroides spengleri	
Paralichthys lethostigmus	

which were taken rarely in the bays, to *Cynoscion nebulosus,* which was rarely taken in the open sea. Similar data for the white trout, *Cynoscion nothus,* have been given by Ginsberg (1929), and data for the sea cat, *Galeichthys felis,* have been given by Lee (1937). Like information on several species collected in 1932 and 1933 was included in a discussion of the effect of shrimp trawls on fish (Gunter, 1936).

Table II shows the numbers of fish by families. The

TABLE II

NUMBERS OF FISH TAKEN BY FAMILIES. THE FIGURES ARE DERIVED FROM TABLE I

Family	Number	Per cent.	Number of species
Sciaenidae	90,418	62.6	5
Otolithidae	12,940	9.0	3
Engraulidae	10,961	7.6	1
Ariidae	7,234	5.0	2
Clupeidae	6,253	4.3	2
Heterosomata	5,959	4.2	6
Carangidae	3,500	2.4	1
Polynemidae	3,219	2.2	1
Trichiuridae	1,510	1.0	1
Stromateidae	1,362	0.9	1
Triglidae	499	0.3	2
Tetraodontidae	272	0.2	1
Ephippidae	257	0.2	1

most successful family in this habitat, from a numerical standpoint, is the *Sciaenidae* and is followed by the closely related family *Otolithidae.* Five species of sciaenids were taken in the trawls. There are other criteria for the success of this family. The species are diverse in size and habits, ranging from the small star-drum, *Stel-*

lifer lanceolatus, to the large, carnivorous *Sciaenops ocellatus* and the oyster-eating *Pogonias cromis.* Species of this family extend from Cape Cod to southern South America and one has invaded fresh water. They are important food fish along the South Atlantic and Gulf states.

The *Ariidae* and *Clupeidae,* represented by two species each, followed the *Engraulidae* in numbers. *Vomer setapinnis* and *Polynemus octonemus,* representing the *Carangidae* and *Polynemidae,* respectively, were summer transients, which is doubtless one reason for their lower positions. The *Carangidae* is a tropical family of diverse, active fishes which is largely absent from the shallow coastal waters during the winter. There are several species of this family in the area, and in many ways it appears to be as successful in its pelagic habitat as the *Sciaenidae* is nearer the bottom.

It is interesting to note that such specialized families as the *Trigilidae* and *Tetraodontidae* were not numerous, although they are very susceptible to capture by trawls.

Table III shows the numbers and percentages of fish

TABLE III

NUMBERS OF FISH CAUGHT IN THE TRAWLS BY ORDERS

Order	Number	Per cent.	Number of species
Nematognathi	7,234	5.0	2
Isospondyli	17,214	11.9	3
Acanthopteri	119,936	83.1	22

taken by orders. As was to be expected, the Acanthopteri are predominant. Apparently the order Nematognathi has been greatly surpassed, yet as species the two catfish representing it, *Galeichthys felis* and *Bagre marinus,* are quite numerous, active and obviously successful. The isospondylous fishes were more numerous than the Nematognathi and are represented by several species, yet the whole order is probably less common than some families of the Acanthopteri, such as the *Sciaenidae* and *Carangidae.*

Anderson (Lindner, 1933) collected similar data by means of shrimp trawls during the years 1930–31 in the inside waters of Georgia. His figures show that 65.3 per cent. of the fish were sciaenids, as compared to 62.5 per cent. sciaenids in Louisiana, but the most numerous species were not the same. In Georgia the star-drum, *Stellifer lanceolatus,* and the spot, *Leiostomus xanthurus,* far outnumbered the croaker. It would appear that these two fish are somewhat better adapted to the waters of Georgia than is the croaker. Variations in abundance from year to year will probably change the picture somewhat. Counts of fish taken on the Mersey shrimping grounds of England by Eccles (Johnstone and Jenkins, 1900) with a beam trawl from thirty-eight to forty-four years ago show that flatfish were most commonly caught.

Pearson (1932) in his description of the offshore winter trawl fishery of Virginia and North Carolina remarks on the peculiarity that pelagic fishes such as the mackerel were often taken. His list of species was surprisingly large. The situation was the same in Louisiana. It is impossible to be sure on the basis of trawl data alone, but the size of the catches in deeper offshore waters indicates that a large number of fish, pelagic species and plankton feeders included, are often within a few feet of the bottom. The top of the trawl was not more than three or four feet above the bottom while in operation, and in this case was lowered and raised by hand so that very few fish could have been captured while the trawl was not on bottom. It would appear that in littoral marine waters the vertical distribution of fish is large if not greatest near the bottom. Further study of this point is desirable.

Pearse (1936) states that the fact that an animal is alive is a certification that it is to some degree successful. Numbers or abundance may be considered as one criterion of success, but each species has an optimum density fitted to its environment, so that a less numerous one may function as well or better than a more common species. Hewitt (1921) and Elton (1927) have pointed out that

great abundance is no surety that a species is in no danger of extinction, and that under adverse conditions it may be wiped out in a few years, even though it be enormously abundant. Many examples may be cited to prove the truth of this observation. Nevertheless, from a statistical and individual standpoint the abundance of a species, that is more or less maintained from year to year, is a fair indication of the past and the inferred future success of a species. When the abundance of a family is coupled with many species of diverse habits it is a good indication that the family is one of the most successful from an evolutionary standpoint and is one of the groups most likely to maintain descendants.

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SHORTER ARTICLES AND DISCUSSION

SOME CONSIDERATIONS CONCERNING THE STRUCTURE OF CHROMOSOMES AND GENES

In a recent paper, Dr. Wrinch (1936) has pointed out several interesting similarities between the microscopic and submicroscopic structure of the chromosome as determined by cytology and genetics, and its chemical structure as revealed by chemical analyses of nuclear material. Her discussion indicates that the linear order of the genes and the capacity of the chromosomes to swell and contract can be explained in large part in terms of chemical facts already available.[1] Dr. Wrinch discusses also the capacity of the chromosomes to grow and to divide, but her explanation of these phenomena does not seem to be mechanically complete or satisfactory.

The capacity of genes and chromosomes to grow and divide and to mutate, and following mutation to reproduce in the mutated form, is an accepted fact of modern genetics, and a fact whose fundamental importance has been stressed by a number of writers (Muller, 1922; Alexander and Bridges, 1928; Gates, 1932; Demerec, 1933, and others). The peculiar interest of this reproductive or autocatalytic capacity lies in its likeness to the fundamental characteristic of living things in general, for it is the capacity of the individuals of the organic world to reproduce themselves, sometimes perfectly, sometimes with more or less variation, which sets them apart from all inorganic things, and which is the basis of their evolution from lower into higher forms. Life, in fact, can be defined as the capacity for mutable reproduction, that is to say, reproduction with the occurrence of occasional transmissible variations. Used in this sense, genes and probably filterable viruses are properly spoken of as alive.

A second phenomenon, almost equally remarkable, which the genes exhibit, is the pairing of homologues prior to the formation of the germ cells. The chromosomes and genes occur in pairs in all body cells, and in certain cells, particularly those involved in the formation of eggs and spermatozoa, the members of a pair unite, like gene pairing with like gene. Of this phenomenon Dr. Wrinch has nothing to say.

[1] See also Hammarsten (1924) for evidence as to the rôle of nucleic acid in chromosome swelling and contraction.

These two attributes of the gene, discussed incompletely or not at all by Dr. Wrinch, have been made the subject of a recent paper by Muller (1937). This paper is primarily a statement of the problem, and a plea to physicists to aid in its solution, but it offers also a partial explanation.

Of the genes' "*specific autoattraction* of like with like" Muller (1937) has the following to say:

> Now, under certain conditions, it becomes evident that each gene forms the center of a specific field of attractive force, for then A tends to come together with the other A, B with the other B (L with L, M with M, and so on). . . . Unlike the ordinary forms of adsorption known to the physical chemists, these gene forces are of such range as to act over visible microscopic distances. In doing so, moreover, they must in some way interpenetrate one another in many directions, since the forces of attraction of many genes must be traversing the same space at the same time.

Muller thus postulates highly specific forces to account for the pairing of homologous genes; forces, moreover, which *act at a distance,* thousands of different kinds of fields of force, one for each of the thousands of different kinds of genes in the plant and animal world. If this is a fair statement of the situation, the biologist may well believe that he has a poser of the first magnitude to place before the brothers in the physical sciences. There are, however, reasons to think that the enigma of gene pairing does not involve as unique or difficult a problem in physics as Dr. Muller's statement would indicate.

There is, in the first place, definite cytological evidence that the pairing of genes is not always completely specific. The evidence comes from cytological observations by McClintock (1932, 1933) and Burnham (1932) on the synapsis (pairing) of chromosomes in corn. Miss McClintock has found "through a study of inversions, deficiencies, translocations and trisomics . . . that non-homologous parts of chromosomes can be intimately associated at the mid-prophase of meiosis in *Zea mays.* This association of threads is similar in appearance to that between homologous parts of chromosomes." Miss McClintock's observations seem to be quite conclusive. She finds that, though in ordinary cases each gene pairs only with its mate or homologue, yet if placed opposite a non-homologue in the gene string, as happens in translocations and other chromosome rearrangements, it may pair with it. The forces involved in pairing are not completely specific. Even so, no one conversant with the facts can deny that

a high degree of specificity exists and that this specificity constitutes a fundamental fact of genetics requiring explanation.

In the second place, there is reason to believe that the pairing, in some cases and perhaps in all cases, is not very intimate, and that it involves a hull of substance that covers the homologous genes rather than the genes themselves. The evidence on this point is primarily cytological. In figures of synapsed (paired) chromosomes drawn by many observers, the appearance is that of two identical and adjacent strings of beads, loosely strung, or of two parallel cobwebs bound together by identical and partly coalesced droplets of viscous liquid (Dark, 1934; Richardson, 1935; Koller, 1936; McClintock, 1931, and many others). It is universally agreed that the droplets or "chromomeres" do not represent the actual genes, but rather hulls of substance surrounding them. The cytological picture, thus, is clearly one of a pairing of shells of substance surrounding the genes and not of the genes themselves. There is visible evidence of a miscibility of homologous shells, but not of a mutual attraction of homologous genes.

In the third place, in many organisms there occurs in meiosis, prior to chromosome pairing, an orientation of the chromosomes which, by bringing homologous chromomeres adjacent to each other, may well render unnecessary as a cause of pairing any force between genes able to "act over visible microscopic distances." This orientation is the so-called "bouquet-stage," represented diagrammatically in Fig. 1 (see also Fig. 274 of Wilson, 1928). It has been described by many cytologists (see, for example, Figs. 270, 271, 272, 279, 280 in Wilson's "The Cell," 1928) and typically consists of the attachment of both ends of every chromosome to one small region of the nuclear membrane, or at least their approximation to this region, the body of each chromosome therefore forming a loop. The orientation of the ends is towards the centrosome (and nucleolus?) and these bodies may be causal agents. Following orientation, pairing of homologues begins at the ends and progresses towards the chromosomes' centers.

With an open and clearly-marked bouquet-stage the process of conjugation advances with considerable regularity from the nuclear pole, nearest the central bodies and idiozome. . . . In cases of this type the spireme-threads are most commonly, and perhaps always, loop-shaped with both free ends directed towards the pole. In either case side-by-side union of threads begins at their

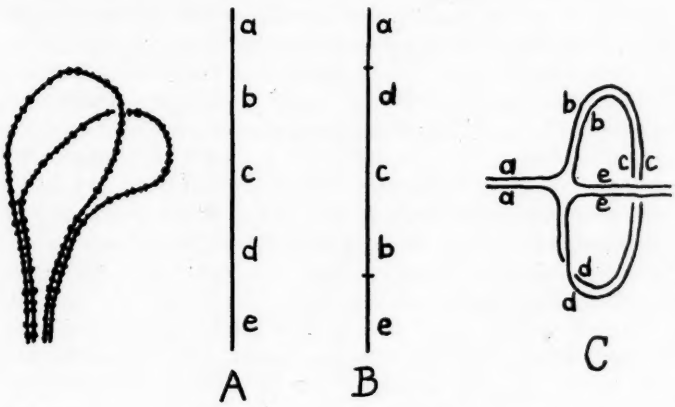

FIG. 1 FIG. 2

FIG. 1. Diagram showing the pairing of homologous chromosomes during the "bouquet" stage. Pairing begins at the two ends which are oriented towards one pole of the nucleus. The bead-like elements are the chromomeres. FIG. 2. Diagram showing synapsis of normal and inverted chromosome. A. Normal chromosome. B. Chromosome with inversion. C. Synapsis of normal and inverted chromosomes. This type of synaptic figure shows the remarkable specificity of pairing. However, McClintock finds that where non-homologous parts of chromosomes are brought together, as happens near the point of breakage in the pairing of inversions, they may be intimately associated. Not infrequently the inverted region fails to pair altogether with its normal homologue. Chromomeres are not shown, but ordinarily would be present in chromosomes in meiosis.

free ends, nearest the pole, and proceeds thence step by step towards the opposite pole. Thus arise characteristic Y-shaped figures, with thick longitudinally double stemps, from which diverge the two halves to form the single branches of the Y. In many cases this process advances with considerable regularity, so that in its middle stages one-half the nucleus is occupied by thick and more or less parallel double threads, the other by single thin and often contorted single threads. . . .[2]

It would be hard to conceive of a contrivance better adapted than the bouquet stage to facilitate pairing. A person wishing to pair two threads first takes the two ends and places them together. Nature, to speak in teleological terms, does this by orienting the chromosome threads in the bouquet. Homologous chromomeres are thereby automatically brought to the same level, and a certain amount of movement or stirring about of the

[2] E. B. Wilson, "The Cell," 1928, pp. 552–553.

threads on the surface towards which they are oriented may be expected to bring homologous chromomeres in contact as a matter of chance without the aid of any special force acting between them. That motion occurs is rendered probable by the great activity of the cell contents as seen in motion pictures of cells grown *in vitro*. The orientation of a single end towards the nuclear pole might seem to be sufficient to insure pairing, but it would have to be the same end in every case, for if one chromosome had one end towards the pole and the other chromosome the other end, pairing would be impossible. Perhaps this is the reason for the loop-like orientation.

In certain forms, particularly most insects and higher plants, a clear bouquet stage is not present, but there is visible instead a contraction-figure or synizesis in which the chromosomes are pressed together in a knot in one region of the nucleus, most commonly the region occupied by the nucleolus (Wilson, 1928, p. 550). This condition may serve, in lieu of a bouquet stage, to aid pairing by reducing the distance between homologous chromomeres. Further, that orientation is entirely lacking in synizesis seems difficult of proof owing to the poor visibility.

While there is excellent evidence of an orientation or packing of the chromomeres favorable to pairing in the early stage of meiosis, yet it also seems to be true that pairing can occur without these. Pairing in the salivary glands of Drosophila of two homologous chromosomes, one of which carries an inversion (Fig. 2), is perhaps the most striking evidence of the specificity of the attraction between homologous genes or homologous chromomeres. However, the importance of some aid to normal pairing is indicated by the fact that in the hemipter, *Euschistus,* in which neither synizesis nor polarization is present, the process of pairing proceeds very irregularly (Montgomery, see Wilson, 1928, p. 552).

In conclusion, it seems doubtful whether the pairing of homologous parts of chromosomes proves the existence of attractions between homologous genes able "to act over visible microscopic distances." It may be that pairing occurs only following the bringing into actual contact of homologous elements. Further, the cytological picture suggests that the pairing elements may be the chromomeres, or a substance surrounding the genes, rather than the genes themselves, and that the tendency to pair, while indeed remarkable in its specificity, is yet such as sometimes to

permit the pairing of non-homologous when these are in contact.

In the present state of our knowledge, an attempt to explain synapsis must involve an entry into the realm of speculation. It seems of interest, nevertheless, to consider certain physical phenomena which present at least interesting parallels to the phenomenon of gene pairing.

The essence of synapsis is an attraction of like for like. This attraction may involve forces acting at a distance between homologous genes, as assumed by Muller, or it may simply involve a miscibility of homologous chromomeres and a relative immiscibility of non-homologues. Such attractions of like for like are not unknown in physics and chemistry.

The most fundamental is the attraction of like electric currents, which is the basis of magnetism. This appears to have no immediate application in the present instance.

An attraction between like molecules is found in crystal formation. A growing crystal gathers to itself from the substrate molecules of its own kind, due, apparently, to a specific attraction of the molecules composing the crystal for the other identical molecules in solution. Again, any relation to synapsis appears problematical, though, as will be suggested later, there is a possible bearing on gene growth.

Finally, there is an attraction of like for like (using "attraction" in a limited sense to exclude forces acting at a distance) in the case of liquids. Every liquid is miscible with itself, but immiscible with certain other liquids, particularly those chemically dissimilar. If the chromomeres consist of specific substances, differing from chromomere to chromomere, then there will be a greater tendency for homologues to fuse or "pair" than for non-homologues to fuse or "pair." This seems to offer a plausible, though obviously a speculative, explanation of chromosome pairing.

The pairing of like chromomeres is not an isolated case in biology. There are numerous other instances of fusion of like with like, some of them almost as striking as that exhibited by the chromosomes.

Perhaps the most remarkable is the fusion of swarms of myxobacteria to form fruiting bodies. The myxcbacteria are a family of rod-shaped bacteria that live in colonies on dung and various decaying substances. During the period of feeding and growth the colonies possess a capacity for slow, creeping motion. Fol-

lowing this period they form characteristic and often very attractive and brightly colored fruiting bodies in dimensions up to the size of a pinhead. The fruiting bodies produce spores capable of giving rise to new colonies. Now if two different colonies are grown on the same agar plate, they are likely to come in contact as a result of their motion over its surface. If they are closely related, they will fuse and continue their growth as a single swarm. If they are unrelated they remain in contact, but a sharp boundary line persists between them, and they form separate fruiting bodies. Wolf (1909; see also Jennings, 1920), in a very interesting study showed that within one strain carried through many transfers there may arise by mutation a number of different substrains no longer capable of fusion. The capacity for fusion is thus highly specific, and the case seems to present an interesting parallel to the high specificity in the pairing of homologous genes.

Other cases of fusion of like with like can be found, for example, the fusion of the two pronuclei, male and female, in the fertilized egg, and the fusion of the spindle fiber regions in the salivary gland chromosomes of Drosophila. In none of these cases, however, is there any reason to assume the existence of forces acting at a distance.

If the force responsible for chromosome synapsis were a force between homologous genes, it might offer a partial explanation of gene growth and reproduction. This point has been made by Professor Muller (1937), who writes:

> The reason why I think there may be a relation between the two properties is this. If the attracting principle of like for like, which we already know to be possessed by the gene considered as a whole, extend also to more elementary parts of the gene, to "blocks" whose differences in arrangement constitute the specific differences in gene pattern whereby one gene differs from another and which form the basis of the mutational changes, then, if we suppose that representatives of these more elementary "blocks" exist in scattered disorganized form in the space surrounding the genes, it can be seen that each gene-part or "block" would tend to attract to itself another, like part, and so a second group of parts would gather next to the original gene in the same pattern as in the latter, in much the same way as, on a still grosser scale, each chromonema as a whole builds up a second chromonema, having its individual genes identical with and arranged in the same order as in the first one. If, then, the auto-attraction holds not merely for genes as a whole but also for gene-parts the auto-synthesis of a gene as a whole would be largely explained in terms of this auto-attraction.

Reasons have been advanced above for believing that there does not exist an auto-attraction between genes of the sort assumed

by Muller. Consequently, autosynthesis of the gene can not be explained in terms of such an auto-attraction. Yet this does not rule out the possibility that there is, as Muller suggests, a specific attraction between the parts of each gene and corresponding blocks present in the surrounding substrate, and that this attraction is the basis of gene growth. It appears worthwhile to consider this possibility further.

The first line of evidence that requires to be considered comes from biochemistry. Genic material can be obtained from sperm head, where chromatin is packed in a dense mass to the exclusion of almost everything else, in purer form than from any other source. In their classic analysis of the heads of fish sperm, Miescher and later Kossel (1928) found them to consist principally of two substances, a nucleic acid and a relatively simple protein which they named protamine. As Dr. Wrinch points out, there is excellent reason for locating "the genetic identity of a chromosome in its characteristic protein pattern." The peptide linkage of the amino acid residues of the protamines giving long chain molecules brings the chemical structure of the protein constituent of chromatin into accord with the known linear order of the genes (Wrinch, 1936). Further, nucleic acid is too simple and uniform to permit it to be identified with the enormously variable units of Mendelian heredity. The gene is thus probably a protein and built up ultimately of amino acids.

A second line of evidence is furnished by cytology. When the chromosomes, or more precisely the chromonemata (Bonnevie, 1908; Kaufmann, 1926; Hedayetullah, 1931; Nebel and Ruttle, 1936, etc.) divide, they do so by longitudinal fission, homologous element separating from homologous element. This means that the daughter gene must have been formed directly beside the parent gene, presumably in actual contact with it. It can hardly be doubted that the parent gene exerts its catalytic influence on the growing daughter gene through this contact. The two chromonemata before splitting may be likened to two identical strips of picture film lying one on the other with like pictures superimposed. Cytological evidence does not rule out the possibility that instead of being merely two strips of "film" there are actually a number, all alike and exactly superimposed; in fact, there are a number of reasons for preferring this assumption. In this case, division would be into two equal groups of "film" when the chromonema splits.

A third line of evidence is furnished by x-ray analysis of various plant and animal products. Perhaps the most pertinent case is that of the cellulose cell wall of plants. X-ray analyses of plant fibers have shown them to consist of layers of long cellulose molecules, with the molecules of one layer directly superimposed on the molecules of the layer beneath (Sponsler, 1929). These cellulose molecules, with the empirical formula $(C_6H_{10}O_5)_n$, are built up ultimately from glucose, $C_6H_{12}O_6$, and water is released in the process. Sponsler suggests that there may be an attraction between the cellulose molecules of the innermost layer of the cell wall and the glucose dissolved in the protoplasm, and that neighboring glucose molecules bound to the cellulose through this attraction are in a favorable position to react with the loss of water, thus forming a new layer of cellulose whose atoms are directly above the corresponding atoms of the previously formed layers.

The similarity of this method of cellulose formation suggested by Sponsler (1929) to the mechanism of gene autocatalysis proposed by Muller (1937) is apparent. If genes are protein in nature, and are surrounded by a substrate containing amino acids or related substances, it is a plausible assumption that each gene attracts to its surface the amino acids (or other "building blocks") necessary for the formation of a daughter gene beside it, each amino acid being held in the proper position for synthesis into a new gene. The attraction of like for like involved is perhaps similar to the attraction of like for like found in crystal growth, and the bonds holding the separate pieces of the new gene to the old may be supposed to be of the same nature as the bonds that hold the molecules of a crystal together. Once bound beside the parent gene, the substrate molecules are in a favorable position to undergo chemical union, with the loss of water or other by-products. The result is a daughter gene bound together by chemical bonds, and held to the parent gene by some weaker type of bond. This process may be assumed to be repeated an indefinite number of times, resulting in a number of genes, or perhaps better "genic layers," superimposed one on another.

Besides the analogy with cellulose, there is a second reason for the assumption of repeated layers. A process of the type assumed presumably would be reversible. The direction in which it would move would depend on the concentration of the substrate. Were only one new gene formed, it might at any given instant, even in

a concentrated substrate, be incomplete, so that division of the chromonema at this instant would result in a daughter gene showing a "loss mutation"; in fact, such genes would probably be the rule rather than the exception. The addition of further layers would protect the inner layers and ensure some of them being complete.

The process assumed is thus partly a crystallization and partly a chemical union, and the resulting gene may be thought of as in part crystal and in part simple protein molecule. The gene, if this be its nature, should show a structure capable of at least partial analysis by x-rays.

It is worthy of note that, in view of the probable protein nature of the gene, a process which explains gene reproduction also explains at least one type of protein synthesis.

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THE CULTURE OF *PECTINATELLA MAGNIFICA* LEIDY

ROGICK,[1] in describing the culture of fresh-water Bryozoa, states that the problem of culturing these animals has been a difficult one. She describes a method whereby organic debris from greenhouse tanks was used successfully to rear colonies of Lophopodella. However, this worker goes on to say that "Lophopodella will tolerate stagnant and polluted water to a surprising extent," while "Pectinatella, Cristatella and some of the other Bryozoa could not tolerate such conditions so well." Brown[2] has been successful in culturing Plumatella by using "Geha" fish food, size 000. He kept the colonies alive and reproducing four weeks.

It has been observed that certain fresh-water Bryozoa, such as Pectinatella and Plumatella, will thrive for a period in an aquarium into which they have been introduced together with water plants collected in the field. After a few weeks, they apparently disappear. Then again after some weeks, the animals reappear and bud prolifically. This cycle may repeat itself time and time again. It may be observed, at this time, that the

[1] Mary Rogick, "Culture Methods for Invertebrate Animals," pp. 179–80. Ed. by J. G. Needham, *et al.,* Comstock Publishing Co., Ithaca, N. Y., 1937.

[2] C. J. D. Brown, *Trans. Am. Micros. Soc.,* 53: 425, 1934.

appearance of the colonies coincides with the presence of debris in the aquarium (*i.e.*, uneaten food, dying plants).

Recently, the present writer had the opportunity to publish in this journal[3, 4] a method for culturing certain Protozoa. Essentially, this method involves the use of finger bowls with an agar base in which rice grains have been imbedded. To this a medium of the following constitution is added:

NaCl	1.20 gms
KCl	0.03
$CaCl_2$	0.04
$NaHCO_3$	0.02
Sorenson's Phosphate buffer pH 6.9–7.0	50 cc
Distilled water to	1000 cc

This is a stock solution; it is diluted 1: 10 for use.

By this method the following animals have been cultured: *Amoeba dubia, A. proteus, Arcella vulgaris, Actinosphaerium eichornii, Spirostomum ambiguuim, S. teres, Stentor coeruleus, Stylonichia notophora, Blepharisma lateritia* and *Paramoecium bursaria.* It has been observed that amoebae cultured on the agar base are not best suited for class use or micrurgy, on account of the agar debris which clings to the pipette. However, in the case of the Amoeba it is just as well to omit the agar; the animals reproduce about as prolifically as those cultured on agar. Since the medium had proved itself an ideal substitute for pond water and since such animals as Cyclops, Cypris, Daphnia, Planaria and Tetrastemma have also responded to certain modifications of this method, it was thought advisable to attempt the culture of fresh-water Bryozoa. The following modification was found to be very successful for *Pectinatella magnifica.*

The animals were collected originally in aquaria inhabited by two species of tropical fish, *Lebistes reticulatus,* the guppy, and *Xipophorus helleri,* the Mexican swordtail. They were found attached to the undersides of leaves of *Vallisneria spiralis* and of *Elodea canadensis,* with which they had probably been introduced. The writer is also indebted to Mr. Hugh E. Potts, a graduate student at New York University, for specimens of Pectinatella from his aquaria.

A finger bowl is thoroughly cleaned and prepared as follows: A quarter inch layer of hot 2 per cent. agar in distilled water is

[3] P. F. Brandwein, AM. NAT., 69: 628, 1935.

[4] P. F. Brandwein and J. A. Cohen, AM. NAT., 70: 429, 1936.

poured into the finger bowl. While the agar is still warm, one half of a Petri dish or a similar container is imbedded in the agar with the inner surface upward. The result is a dish within a dish, the inner one being fixed by the agar which forms a ½ inch margin around it. In this warm agar margin, fifteen equally spaced grains of wheat are imbedded. The agar is permitted to cool. Then enough of the solution is poured in so that the fluid is about one third of an inch higher than the rim of the inner dish. This is inoculated with Chilomonas or Copidium, or both, and allowed to stand for two days. At this time, a portion of a colony of *Pectinatella* together with 20 cc of the water in which the animal is found, are introduced into the inner dish. Three to five zooids dissected away from the parent colony are sufficient for inoculation. The finger bowl should not be disturbed for twenty-four hours so as to permit the animals to fix themselves to the glass. Development is rapid; a few hundred zooids are usually developed within two to three weeks; statoblasts are very numerous. Subculturing is not difficult. Usually, after three to four weeks, it will be found that the colony has broken up and that small clumps of two to three individuals may be found. These small colonies may be used for inoculation after they have been scraped from the glass.

The colony will feed on Paramoecium, Blepharisma, Arcella and Monostyla. In fact, the introduction of these animals into the culture results in the more rapid growth of the colony. The smaller zooids, however, sweep in such animals as Colpidium and Chilamonas.

After four weeks, there appears a gelatinous coating over the bottom of the dish; this usually covers the colonies. When this occurs, it is advisable to subculture or remove this gelatinous coating. At times, the population of Protozoa makes the medium cloudy. When this occurs, one half of the fluid may be removed and replaced with fresh solution.

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